ABSTRACT

NIFONG, JESSICA MARIE. Assessment of the Diversity and Disease Resistance Properties within a Collection of *Nicotiana rustica*. (Under the direction of Jennifer Nicholson and Ramsey Lewis.)

Nicotiana rustica L. (2n = 2x = 48) is one of numerous natural amphidiploid species within the genus *Nicotiana*. This species exhibits high levels of phenotypic variation, most likely resulting from genome reorganization and homogenization since speciation. To assess the phenotypic and molecular diversity present within *N. rustica*, phenotypic data was recorded for 86 diverse accessions over two field seasons and the *trnL-F* chloroplast intergenic region was sequenced for each accession. Due to the lack of intraspecific variation within this region, amplified fragment length polymorphism (AFLP) markers were used to estimate the genetic diversity among the *N. rustica* accessions. Principle component analysis of the morphological data revealed a continuum of variation, with an average phenotypic distance between accessions of 0.346 (DGOWER, SAS). Genetic data was subjected to sequential agglomerative hierarchical nested cluster analysis (SAHN), neighbor-joining cluster analysis and STRUCTURE analysis. The average genetic distance (Jaccard's) between pairs of accessions was 0.281. The output from these three clustering analyses failed to display substantial convergence and there was little evidence to support a phenotypic correlation between members of clades or populations. However, a Procrustes analysis performed on the non metric dimensional scaling configurations of a reduced phenotypic data set and the genetic data set revealed a significant correlation (r = 0.2101) between the phenotypic and genetic data.

Losses due to disease can have a major impact on the overall yield of a tobacco crop. Resistance to few diseases has been found within tobacco germplasm, and thus wild *Nicotiana* species have been extensively screened for sources of resistance. *Nicotiana rustica* is among those species that have been previously evaluated as potential sources of disease resistance traits that could be introgressed into commercial tobacco (*N. tabacum*). It had been concluded that *N. rustica* is resistant or tolerant to black shank (caused by *Phytophthora*

nicotianae var. *nicotianae*), *Tobacco Mosaic Virus* (TMV), and wildfire (caused by *Psuedomonas syringae* pv. *tabaci*). In these previous studies the diversity within this species was ignored as only one or a few accessions of *N. rustica* had historically been used during disease screening. In this study, 86 diverse accessions were screened for variation in pathogen response that may have been overlooked in previous studies. Though experimental inoculations confirmed that *N. rustica* is indeed generally susceptible to many pathogens, responses were not always uniform across or within accessions. All accessions were found to have a hypersensitive response type of resistance to TMV. Additionally, all accessions were highly resistant or immune to black shank race 0. There was a large degree of variation in responses to race 1, however, ranging from susceptible to highly resistant. Hybrids between *N. rustica* and *N. tabacum* were generated by using tetraploid K326 as the male parent and were subsequently backcrossed to K326. Resistance to TMV has been carried successfully into the second backcross but more studies are needed on the transmission of race 1 black shank resistance.

Assessment of the Diversity and Disease Resistance Properties within a Collection of *Nicotiana rustica*

by
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A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Crop Science

Raleigh, North Carolina

2008

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AKNOWLEDGEMENTS

I would like to thank the NC Tobacco Foundation for providing the funding for this research, as well as the R. J. Reynolds Tobacco Company and the Lorillard Tobacco Company for their fellowships. I would like to thank my committee members, Jennifer Nicholson, Ramsey Lewis and David Shew, for their direction and advice during the course of this project. I would also like to thank Ignazio Carbone and Consuello Arellano for helping me to understand some of the complex analyses of my genetic and morphological data. I am deeply in debited to Neal Tobias who completed much of the preliminary activities of this project, including production of the interspecific hybrid seed. I could not go without thanking Cara Rose for her patience in answering my endless questions about “plant things” I did not yet understand. And although they may never see this, I would additionally like to thank all of the excellent professors I have had over the years, both here and at UNC-CH. It is their passion for education that has led me to reach further in my own pursuit of knowledge.

BIOGRAPHY

Jessica Marie Lott was born on May 31, 1983 in Stuart, Florida. There she had a happy childhood annoying her older brother, watching the Discovery Channel with her grandfather, and crafting with her beloved grandmother. She was always very interested in nature and being outdoors. Although her family moved around quite a bit, it never impacted her studies. She graduated from South Johnston High School in 2001, where she is most known for taking a previously unheard of seven science classes within her three years of attendance. She remembers fondly her senior project, "Transgenics for Dumbies", a how to guide for transforming plants using *Agrobacterium*.

Jessica had the good fortune of being accepted to the only college she applied to, UNC-Chapel Hill, because she did not have a backup plan for what she was going to do if she did not get in. While she was there she indulged herself in all sorts of interesting topics, from philosophy to astronomy, to marine ecology and microbiology. Working in a yeast genetics lab under Dr. Kerry Bloom provided her with valuable molecular biology skills, but probably more important at the time—cash for her empty pockets. She graduated from UNC in May of 2005 with a bachelor's degree in biology with a minor in chemistry.

After many months of doing painstaking retail work while waiting for calls about interviews that never came, she decided it was time to go back to school. So falling back on her passion for plant transgenics, Jessica applied to NC State because, well, they don't do plants at UNC. She was accepted to the Tobacco Breeding, Genetics and Molecular Biology program in the Department of Crop Science and began attendance in January 2006. While in attendance she was finally married to her long time sweetheart, Thomas Nifong. Though she doesn't think that her new name, Jessica Marie Nifong, sounds as good, she wouldn't change it back for the world.

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Chapter One

Literature Review

*N*ICOTIANA RUSTICA L.

Description and History

N. rustica L. (2n = 4x = 48) was first described by Carolus Linnaeus in 1753 based on a sample simply stated as from "America", although it is believed to have originated on the western slopes of the Andes Mountains in Peru or Ecuador (Goodspeed, 1954). Kostoff (1943) described *N. rustica* as an herbaceous annual "60-180 cm high [with leaves] 6-45 cm long and 2-35 cm broad, depending on the variety and on the external conditions." The leaves are petioled and may be elliptic, ovate or cordate in shape, depending on the variety and location on the plant. Foliage is typically dark green and the stems can be smooth or ridged. Flowers are five lobed with yellow green corollas approximately 12-17 mm long (Goodspeed, 1954).

Referred to widely as Aztec tobacco, *N. rustica* was grown by Native Americans as a medicinal herb and it is believed to have been traded from South American tribes northward. According to Goodspeed (1954), it became commonly cultivated in Mexico, the southwestern and eastern United States and eastern Canada by the time of the arrival of the first European explorers. It is believed to have been the first form of tobacco grown and exported to Europe by the American colonists. Its cultivation was gradually replaced by the higher yielding *N. tabacum* L., although *N. rustica* remained in cultivation as the "peasant tobacco of Central Europe and western Asia" (Goodspeed, 1954). The species is now pandemic due to its historic growth for smoking and its later cultivation for the extraction of nicotine and citric acid (Kostoff, 1943).

Early cytogenetic studies by Kostoff (1943) and Goodspeed (1954) determined that *N. rustica* is an allopolyploid resulting from the natural hybridization of two diploid *Nicotiana* species, most likely *N. paniculata* (2n = 2x = 24) and *N. undulata* (2n = 2x = 24). Lim *et al*. (2007) report that this hybridization most likely occurred less than 200,000 years ago, though more evidence for this is forthcoming (publication in process, J. J. Clarkson, personal communications). In another study, Lim *et al.* (2005) used fluorescent *in situ* hybridization

(FISH) to successfully paint *N. rustica* chromosomes with probes derived from the genomes of *N. paniculata* and *N. undulata*, supporting the role of these two species in the formation of *N. rustica*. This link is also weakly supported by the analyses of isozymes completed by Smith *et al.* (1970) and Reddy and Garber (1971), which both reported similarities between the peroxidase and esterase profiles of the allopolyploid species and its proposed diploid progenitors.

However, there has been recent speculation over the progenitors of *N. rustica*. With the increase in the accessibility of sequencing technology, it has been shown that *N. rustica* may actually be more closely related to *N. knightiana* than to *N. paniculata*. Evidence for this comes from the phylogeny presented by Akoi and Ito (2000), based on the sequences of the chloroplast gene *matK*, in which there are fewer steps (mutations) from *N. rustica* to *N. knightiana* than to *N. paniculata*. Clarkson *et al.* (2004) sequenced three additional plastid loci and also reported a smaller total number of mutational differences with *N. knightiana*. In both studies, *N. rustica* was shown to have diverged prior to the *N. paniculata*/*N. knightiana* pair which makes it more likely that it was the most recent common ancestor of these sister species that was the maternal progenitor of *N. rustica*. The same phylogenetic signal was also detected from nuclear loci by Chase *et al.* (2003) when analyzing three nuclear ribosomal DNA loci within *Nicotiana*.

Within the genus, Goodspeed (1954) considered *N. rustica* to be second only to *N. tabacum* in its level of phenotypic variation. In fact, the diversity was so apparent that *N. rustica* was often the model species of choice for J. L. Jinks when attempting to divulge early quantitative genetic theory (Jinks, 1954; Perkins *et al.*, 1971; Pooni *et al.*, 1978). Several researchers, such as Comes (1899), have historically worked to classify accessions into intraspecific varieties based on this variation. While Danert (1963) recognized six varieties, Goodspeed (1954) only included three in his work, though he noted that they were included only to represent the extremes along a continuum of variation. Accessions show varying levels of characteristics derived from the progenitor species, such as in type and distribution of trichomes (Goodspeed, 1954), level of leaf surface puckering or presence of ridged stems (personal observations). These varying combinations and levels of traits could be the result of considerable chromosomal rearrangement since the time of divergence, as was demonstrated to have occurred in the 18-5.8-26S rDNA locus by Matyasek *et al.* (2003).

N. rustica has been the subject of scientific investigations for hundreds of years. It was reported to be crossed to *N. paniculata* in 1760 by Kölreuter (1761) in his studies on the reproduction of plants. It is believed that this is the first account of an interspecific cross within the genus *Nicotiana*. This particular cross received much attention during the years that followed as it was also made by Gärtner, Naudin, Godron, Hedwig and Wiegmann, all of whom additionally crossed *N. rustica* in various combinations to numerous other *Nicotiana* species (see Kostoff, 1943 for references to these materials). Later, with the rediscovery of Mendel's Laws, scientists focused their research on determining the inheritance of certain *N. rustica* morphological characters, such as seed coat color (East, 1928), and in depth cytogenetic analyses.

Much of the research conducted on *N. rustica* has been focused on its relatively high alkaloid composition. Nicotine, one of the alkaloids found within *Nicotiana* species, has long been known to be an insect specific neurotoxin and it was commonly used in agriculture as an insecticide. *N. rustica* was a prime target for research in this area because, among its alkaloids, it almost exclusively produces nicotine and the levels can become quite high under the proper conditions. As early as the 1920's, researchers were actively selecting within populations of *N. rustica* for increased nicotine with the specific goal of harvesting it for use as insecticide (Haley *et al.*, 1924). In Virginia, Smith and Bacon (1941) successfully used intraspecific hybridization and selection to increase plant height, leaf number and largest leaf area in *N. rustica*. The increase in size did lead to an increase in overall nicotine yield; however, the percent alkaloid content of the plants was negatively affected by the increase in biomass. The average alkaloid content of the untopped parents ranged from 2.71% to 6.15% while the larger derived lines had only 3.39% to 5.97% (percent dry weight) alkaloids. Woodmansee *et al.* (1944), working in Kentucky, used different fertilizer regimens and found nicotine to range from 6.55% to 8.76% in the leaves of *N. rustica* grown in the greenhouse but, due to the strong impact of the environment on alkaloid biosynthesis, could not reach similar levels under field conditions. (See McMurtrey *et al.*, 1942, for data on *N. rustica* alkaloid content over 14 locations.)

Disease Resistance Properties

Another large body of research involving *N. rustica* pertains to its response to infection with various pathogens. There are many pathogens of commercial tobacco which can have potentially severe impacts on yield. However, resistance to few of these pathogens has been found within *N. tabacum* germplasm. For this reason, the other species of the genus have been heavily screened for resistance to many of the pathogens of tobacco. Below is a summary of how *N. rustica* reacted to a number of tobacco pathogens, beginning with those to which it was susceptible and ending with those to which it was resistant or tolerant.

Frogeye leaf spot (*Cercospora nicotiane* Ellis & Everh.)

Raeber *et al.* (1963) reported no resistance within *N. rustica* but no account was given of the degree of susceptibility among the 13 varieties examined, nor whether conclusions were based on seed bed or greenhouse results.

Brown spot (*Alternaria alternata* (Fr.:Fr.) Keissl.)

Thirteen varieties were rated as susceptible by Raeber *et al.* (1963) but they did not give clarification as to whether this was the result of the seedbed trials or the subsequent greenhouse trials, which made use of humidity chambers for as many as 7 days. In an additional study by von Ramm and Lucas (1963), four varieties were infected, either slightly or more commonly severely, in greenhouse experiments. Mature plants were inoculated, however, they were first dusted with flour and then placed in a humidity chamber for 6 to 10 days after inoculation—conditions which would never be mimicked during natural field conditions.

Anthracnose (*Colletotrichum destructivum* O'Gara)

Raeber and Cole (1963) reported that 11 varieties of *N. rustica* were severely infected during their seedbed experiments. Accessions TW116, TW117 and TW118 were examined by Sievert (1972a) and determined to be susceptible during his greenhouse experiment, but not as severely as the members of Section Acuminatae (*N. attenuata*, *N. corymbosa*, *N. linearis*, *N. miersii*, and *N. puciflora*).

Root-knot nematodes (*Meloidogyne javanica* (Treub) Chitwood)

Clayton *et al.* (1958) reported a disease index representing the percentage of root infection as 86, making *N. rustica* highly susceptible. The species was also reported as susceptible by Schweppenhauser *et al.* (1963).

Granville or bacterial wilt (*Ralstonia solanacearum* (Smith) Smith)

Clayton and Smith (1942) simply reported *N. rustica* as "highly susceptible".

Tobacco Streak Virus

Diachun and Valleau (1954) explicitly mentioned *N rustica* as the systemically infected virus stock plant used to maintain their virus stock but additionally noted that it had moderate streak with "little or no stalk necrosis".

***Tobacco Etch Virus* (TEV)**

N. rustica was reported by Holmes (1946) as developing systemic infection with vein clearing and chlorotic mottling when infected with TEV, however during inoculations he wounded each plant with 100 pin pricks.

***Tomato Spotted Wilt Virus* (TSWV)**

Gardner and Whipple (1934) reported *N. rustica* as a host for TSWV because it was subsequently infected after being rubbed with a TSWV infected tomato leaf.

Potyviruses

Though he did not mention which strain of *Potato Virus Y* (PVY) he used, Sievert (1972b) inoculated five plants each of TW 116, TW 117 and TW 118 and reported that all had abnormal appearance after inoculation. In another study conducted by van Dijk and Cuperus (1989), the five TW accessions of *N. rustica* were inoculated with two to three isolates of each of the potyviruses *Potato Virus A* (PVA), *Potato Virus X* (PVX), PVY^C, PVY^N and PVY^O. Only a single plant of each accession was tested per virus isolate. They noted some tolerance and resistance to all isolates except PVX, but the temperature was higher in the greenhouse during this test. There was the greatest variability in response to PVY^O, ranging from susceptible to resistant depending on the particular isolate and accession.

Blue mold/Downy mildew (*Peronospora tabacina* D.B. Adam)

The literature regarding the response of *N. rustica* to this pathogen is littered with contradicting reports. In 1934, Clayton and Gaines reported *N. rustica* as resistant but in the same year Wolf *et al.* reported it as susceptible even though they did not actually conduct a test. Smith-White *et al.* (1936) screened 22 varieties, including "*N. campanulata*" (TR 50), and found *N. rustica* to have a wide range of tolerance, though all varieties were more tolerant than *N. tabacum*. However, they note that this resistance would not be useful in a breeding program because it is not as good as that found in the Australian species.

Clayton continued to investigate resistance to blue mold and in 1945, after conducting plant bed experiments from Georgia north to Virginia, revised his earlier reports and scored *N. rustica* as moderately resistant, with only a slight amount of infection. He further reported that the age and vigor of the plants affected the results such that, by 35 days of age, *N. rustica* had developed sufficient levels of resistance to reduce pathogen sporulation, but that plants were very susceptible before then.

In another study conducted in Australia, Wark (1963) screened 26 varieties under artificial conditions and reported no resistance among any of the *N. rustica* accessions. The procedures during this experiment involved inoculating 6 week old seedlings and then covering them to retain high humidity (82-100%) for a week.

They were then uncovered and observed for another week. Interestingly, Wark did not report any tolerance in *N. longiflora* or *N. plumbaginifolia* with these procedures although they had been rated highly resistant to immune under plant bed conditions by Clayton (1945).

Powdery mildew (*Erysiphe cichoracearum* DC)

Raeber *et al.* (1963a) reported that three of eleven varieties of *N. rustica* tested "were visually rated as susceptible" but that limited mycelial growth and sporulation was visible under the microscope even on the eight resistant varieties. Plants were inoculated by natural means inside a greenhouse due to the high prevalence of the pathogen in Southern Rhodesia (Zimbabwe) at that time. Natural infections, in either a greenhouse or a cheesecloth tent environment, were also utilized in the South African study reported by Rossouw (1963). However, in these experiments *N. rustica* was scored as very susceptible. Rossouw noted that differences in responses reported by different studies could be due to varietal differences of the plant species examined or to different physiological races of the pathogen.

Wildfire (*Psuedomonas syringae* pv. *tabaci* (Wolf and Foster) Young *et al.*)

Like with blue mold, reports on wildfire resistance are also contradictory. However, since there are reports of the transfer of resistance from *N. rustica* to *N. tabacum*, *N. rustica* is commonly thought to be resistant to wildfire (Woodend and Mudzengerere, 1992). Johnson *et al.* (1924) reported the successful infection of *N. rustica* but induced wounds in plants to be inoculated by puncturing them with needles. They noted that successful infections were more often procured from inoculations involving needle punctures than when wounds were not present. On the other hand, Anderson (1925), who conducted seed bed experiments in the Connecticut Valley, determined *N. rustica* to be resistant. He reported that eleven "sowings were made with the seven varieties and all were thoroughly inoculated just as the other species of *Nicotiana*, but, in striking contrast to the varieties of *N. tabacum* growing all about, no wildfire appeared on any of them [*N. rustica*]. On the thousands of plants inoculated during the two years perhaps a dozen wildfire spots have been found and these developed hardly at all." Anderson did not artificially wound the plants investigated or manipulate the humidity.

Two other studies report *N. rustica* as susceptible. Diachun and Valleau (1954), who poured bacterial suspension onto the lower leaf surfaces of the plants they investigated, reported *N. rustica* as having "more necrosis and less halo than on tobacco". Raeber *et al.* (1963b) inoculated their plant beds by spraying the bacterial suspension at 70 psi with the nozzle held close to the leaves of plants two months after sowing. They scored *N. rustica* as 42% resistant.

Tobacco cyst nematode (*Globodera tabacum solanacearum* (Miller and Gray) Behrens)

The only information on this pathogen comes from Lownsbery (1953) and he reported *N. rustica* as a poor host with only 7 mature females present, while the *N. tabacum* varieties tested ranged from 23 to 68 mature females.

Black shank (*Phytophthora nicotianae* Breda de Haan var. *nicotianae* G.M. Waterhouse)

The first reports on this pathogen in the United States come from Tisdale and Kelly (1926) in Florida. Out of 54 *N. rustica* plants in the field, 30% became infected late in the season while there was in all practicality a complete loss of the other 18 varieties of cigar wrapper and bright leaf tobacco tested. Also, upon examination of those *N. rustica* plants that were infected, it was discovered that the roots were also infected with root knot nematodes. They noted that it was possible that the pathogen was able to infect *N. rustica* only after the roots had been sufficiently damaged by the nematodes. Foster (1943) also reported that *N. rustica* "showed definite resistance" during his studies.

However, Diachun and Valleau (1954) described *N. rustica* as susceptible but failed to provide any further information. In a study conducted by Lautz (1957), six varieties of *N. rustica* were inoculated in thumb pots at five to six weeks of age where they had 15-100% survival. They were then moved to 4 inch pots and had 0-75% survival after 2-3 months of exposure to a heavy pathogen load. In these experiments the variety pumila performed the best. Litton *et al.* (1970) performed the first race specific black shank test of many *Nicotiana* species. They tested only two varieties of

N. rustica and reported moderate to high resistance for race 1 and high susceptibility to race 0. However, *N. rustica* was recently reported to be susceptible to race 1, with 0-32% healthy plants after infection depending on the variety (Li *et al.*, 2006).

Tobacco Mosaic Virus **(TMV)**

Although *N. rustica* was not considered resistant at the time, there are pictures of its hypersensitive lesions from 1929 where Holmes was testing the efficacy of different TMV inoculation procedures. Later, Holmes (1936) transferred the TMV localizing gene (*N*) from *N. rustica* to *N. paniculata* by backcrossing progeny which localized the virus in necrotic lesions to *N. paniculata*. Despite this, Holmes again classified *N. rustica* as susceptible in his 1946 study, though he observed brown necrotic spots with pale centers at the inoculation site with a systemic spread of necrotic spots. It is worth mentioning that this is the exact same susceptible description he gave for *N. glutinosa*, but his inoculations did involve both rubbing the inoculum on the leaves and 100 pin pricks. These large wounds could have masked the protection of the hypersensitive responses of both species.

Diachun and Valleau (1954) also described *N. rustica* as having "local necrotic spots or blotches, often followed by systemic necrosis" upon inoculation with TMV but this description was additionally given for *N. glutinosa* and *N. paniculata*. Local necrotic spots were also noted on *N. undulata* but were not accompanied by systemic infection. This is important because it shows that *N. rustica* may have received genes for TMV resistance from both of its progenitors. TW 116, TW 117, TW 118 and TW 120 were reported as susceptible by van Dijk and Cuperus (1989) but they noted in the procedures that the temperature in the greenhouse reached as high as 30°C during TMV tests and the *N* gene for the hypersensitive response to this virus is known not to be effective under such conditions (Whitham *et al.*, 1994). This can be seen by the fact that van Dijk and Cuperus also report only one of three *N. glutinosa* accessions, the source of the *N* gene, as displaying a hypersensitive response.

Production of Hybrids with N. tabacum

N. tabacum × *N. rustica*

Swaminthan and Murty (1957) discovered that the cross does not work in this direction due to the failure of *N. rustica* pollen to grow more than a third of the way down the *N. tabacum* style, with frequent tube bifurcation and bursting. Additionally, they discovered that the rate of pollen tube growth in *N. rustica* is slower relative to *N. tabacum*. In another study, Swaminthan and Murty (1959) tried to stimulate additional growth of the *N. rustica* pollen tubes by irradiation with x-rays. With an exposure of 9600 r of x-rays, the length of the *N. rustica* pollen tube was able to increase to 900 microns from 400 microns when not exposed to x-rays. A small number of seeds were obtained when *N. rustica* pollen was irradiated by 4800 or 9600 r of x-rays and then applied to *N. tabacum* pistils. However, the researchers did not report on the ability of these seed to germinate.

In order to determine which progenitor genomes contributed the genetic basis for the incompatibility, Kuboyama and Takeda (2000) pollinated *N. tabacum* and its proposed maternal progenitor species, *N. sylvestris*, with pollen from *N. rustica* and its speculated progenitors, *N. paniculata* and *N. undulata*. They found that when either *N. tabacum* or *N. sylvestris* was pollinated with *N. undulata*, the winding type of incompatibility was observed in the pistilate parent, while it was not present when *N. paniculata* was used as the pollen source. For this reason they concluded that the *N. rustica* genome responsible for the incongruity was derived from the *N. undulata* genome. The corresponding genome (S or T) of the pistilate parent, *N. tabacum*, responsible for the incompatibility was not determined by Kuboyama and Takeda.

However, this interspecific cross can be performed in this direction by manipulating the system. Swaminthan and Murty (1957) reported that capsules were successfully set after cutting the *N. tabacum* pistil to approximately 3 mm and placing agar-sucrose medium on the tip of the cut style before applying *N. rustica* pollen. Cutting the *N. tabacum* pistils allowed the *N. rustica* pollen tubes to successfully reach the ovary. This indicates that style length is the dominant factor responsible for the normal failure of this cross. However, only a few seed were set even when pollinations were successful, suggesting the existence of additional sterility barriers.

N. rustica × *N. tabacum*

There has also been much difficulty in generating hybrid seed from the cross performed in this direction. Where large differences in style length was a somewhat obvious hurdle in the cross *N. tabacum* × *N. rustica*, the reason for the failure of the reciprocal cross, *N. rustica* × *N. tabacum*, is still not understood. Believing post-zygotic sterility barriers were the cause of the failure, Douglas *et al.* (1983) performed an experiment to try to rescue, *in vitro*, ovules that were fertilized *in vivo*. *N. rustica* flowers were pollinated with pollen from *N. tabacum* and then harvested three days later. Either the placentae or individual ovules were removed from the *N. rustica* ovaries and placed on culture medium at 25°C with continuous light. Seeds successfully developed *in vitro* and germinated to produce true hybrids. When assuming that there are 300 ovules per placenta in *N. rustica* (Goodspeed, 1954), successful development of fertilized ovules occurred in this study with only a frequency of 6-11%. Though this is low, it is still orders of magnitude greater than that of non-rescued ovules. Similar results were also reported by Shizukuda and Nakajima (1982) from their *in vitro* culture of individual ovules extracted five days after pollination.

Chaplin and Mann (1961) introduced a breeding technique to overcome the incompatibility barriers encountered in the process of trying to generate interspecific hybrids in the genus *Nicotiana*. The technique involves doubling the chromosome number of one of the parents of the cross by treatment with colchicine. *N. tabacum* was always the autotetraploid parent in their experiments and it greatly improved the efficiency of hybrid production. In regards to producing hybrids between *N. rustica* and *N. tabacum*, they found that even when using tetraploid tobacco, the cross would only set when *N. rustica* was the pistilate parent. The fertility of the cross had been greatly improved by the use of tetraploid tobacco such that, after 20 pollinations, 51% of the seed from tetraploid crosses produced vigorous hybrids (55 hybrids from 107 seeds) while none were produced from the 84 seeds obtained from the diploid crosses. The *N. rustica-N. tabacum* hybrids had the greatest percent survival of any of the other 23 interspecific hybrids generated during the study. Some of their pollen stained but no seed were set when self-pollinated. However, the interspecific hybrids demonstrated low female fertility when backcrossed with diploid tobacco.

The two methods presented differ in the expected chromosome numbers of the progeny. The use of tetraploid tobacco by Chaplin and Mann (1961) increases the chance that one whole copy of the tobacco genome (2n), along with one half of the *N. rustica* genome (n), will be present in the progeny. This leads to a total of 72 chromosomes and a ploidy level referred to as sesquidiploid. In the ovule rescue method (Douglas *et al.*, 1983), the progeny produced are true interspecific hybrids with a haploid genome from each parent resulting in 48 total chromosomes. Each of these types of progeny can be useful. The sesquidiploid state is especially useful in breeding due to the presence of the whole tobacco genome. The chromosomes from the other parent do not pair during meiosis and can thus be shed very quickly. A good selection program can be used to maintain the chromosome of interest while the undesirable donor DNA is quickly eliminated.

Somatic Cell Hybridization

Another method that results in the production of true hybrids is somatic cell hybridization. In plants, this involves the chemical fusion of protoplasts derived from cell cultures of each parent. Researchers in Japan (Nagao, 1978), the United Kingdom (Hamill *et al.*, 1985) and Canada (Douglas *et al.*, 1981a and 1981b; Douglas *et al.*, 1981) have used this method to successfully develop *N. rustica-N. tabacum* hybrids. A difference between this method and the previously described methods is that the hybrids generated from protoplast fusion are expected to be diploid (2n = 96). However the chemical fusion of protoplasts and the cell culture system itself are not genetically conservative. Chromosomes are frequently lost during protoplast fusion and cell culturing commonly leads to mutations. Douglas *et al.* (1981) reported only one *N. rustica-N. tabacum* hybrid out of a total of 28 with 96 chromosomes while Nagao (1978) reported hybrids with 60 to 91 chromosomes.

Cytoplasmic Male Sterility and Advancing Generations

In almost all of the literature on successful *N. rustica-N. tabacum* hybrid productions, the hybrids were described as being male or pollen sterile (see Nagao, 1978, and Hamill *et al.*, 1985, for exceptions). Limited female fertility was always retained, however, and subsequent generations were developed by backcrossing the hybrid to either parent. The cause of no or poor male fertility is due to cytoplasmic male sterility (CMS). This is caused by the presence of factors in the *N. rustica* cytoplasm that interact unfavorably with the *N. tabacum* genome resulting in abortive pollen. *N. rustica* is believed to have cytoplasm similar to that of *N. paniculata* or *N. knightiana* and both of these species have also been reported to produce CMS in combination with the *N. tabacum* genome (Nikova *et al.*, 1991; Berbeć, 2000). As the cytoplasmic factors are genetically controlled, the *N. undulata*-derived genome of *N. rustica* may also contribute to its production of CMS because *N. undulata* has also been reported to produce CMS (Gerstel, 1980). These sterility hurdles are usually overcome by loss of the chromosomes responsible by the third backcross to *N. tabacum* (Legg and Mann, 1961; Pandeya *et al.*, 1986). When working to advance generations after somatic hybridization by self pollinating, early selection for pollen viability did not result in a substantial gain of male fertility (only 16% self fertility during the R_2 generation at best) and required much more resources than traditional backcrossing (Hamill *et al.*, 1985).

Interested in the agronomic effects of incorporating *N. rustica* germplasm into commercial tobacco, Legg and Mann (1961) generated *N. rustica* × *N. tabacum* hybrids following the procedures of Chaplin and Mann (1961) and backcrossed them to diploid tobacco three times without any selection other than the early requirement of descent female fertility. The resulting BC_3S_1 families were compared morphologically and chemically to their parents based on field trials. The majority of lines rated equivalent to their tobacco checks, but transgressive segregants were seen both in favorable (lower number of suckers per plant and increased number of leaves per plant) and unfavorable (increased plant height and increased days to flower) characters. However, during the course of backcrossing, they steeply cut back the population each generation and thus it is possible that transgressive segregants could have been produced for more traits.

Despite the difficulty of generating and working with interspecific hybrids between *N. rustica* and *N. tabacum*, a number of tobacco cultivars have been released containing unquantified amounts of *N. rustica* germplasm. A number of varieties developed in Canada, such as Nordel (Pandeya and White, 1981), Delgold (Pandeya and White, 1984), Newdel (Pandeya *et al*., 1984) and Candel (Pandeya, 1986), all began as *N. rustica* × *N. tabacum* hybrids which were generated by the method of Chaplin and Mann (1961). Another Canadian cultivar, Delfield (Pandeya *et al*., 1991), began as a hybrid produced via protoplast fusion. There are even reports of the successful transfer of resistance traits from *N. rustica* to *N. tabacum*, including wildfire (Stavely and Skoog, 1976; Woodend and Mudzengerere, 1992), blue mold and black root rot resistance (Pandeya *et al*., 1986).

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CHAPTER TWO

Assessment of the Phenotypic and Molecular Diversity within a Collection of *Nicotiana rustica*

ABSTRACT

Nicotiana rustica L. (2n = 2x = 48) is one of numerous natural amphidiploids within the genus *Nicotiana*. The species exhibits high levels of phenotypic variation and has been shown to have undergone genome reorganization and homogenization since speciation. To assess the phenotypic and molecular diversity present within *N. rustica*, phenotypic data was recorded for 86 accessions over two field seasons and the *trnL-F* chloroplast intergenic region was sequenced from each accession. Due to the lack of variation in this region, amplified fragment length polymorphism (AFLP) markers were used to estimate genetic diversity among the accessions. Principle component analysis of the morphological data revealed a continuum of variation and the average phenotypic distance between accessions was 0.346 (DGOWER, SAS). Genetic data was subjected to sequential agglomerative hierarchical nested cluster analysis (SAHN), neighbor-joining cluster analysis and STRUCTURE analysis. The average genetic distance (Jaccard's) between pairs of accessions was 0.281. The output from these analyses failed to display convergence and there did not appear to be any phenotypic correlation between members of clades or populations. However, a Procrustes analysis performed on the non metric dimensional scaling configurations of a reduced phenotypic data set and the genetic data set revealed a significant correlation of 0.2101.

INTRODUCTION

Besides *Nicotiana tabacum* L., *Nicotiana rustica* L. (2n = 4x = 48) is the only other widely cultivated member of the genus *Nicotiana*. Although the species is believed to have originated on the western slopes of the Andes Mountains in Peru and Ecuador (Goodspeed, 1954), it is now pandemic due to its historic cultivation for smoking and extraction of nicotine and citric acid (Kostoff, 1943). This dissemination has resulted in many common names such as Aztec tobacco, mapacho and mahorka. Early cytogenetic studies by Kostoff (1943) and Goodspeed (1954) determined that *N. rustica* is a natural allopolyploid whose most likely progenitors are the diploid species *N. paniculata* (2n = 2x = 24) and *N. undulata* (2n = 2x = 24). This was supported by Lim *et al.* (2005) when they used both genomic and fluorescent *in situ* hybridization to successfully paint *N. rustica* chromosomes with probes derived from *N. paniculata* and *N. undulata*. However, based on the total number of mutations from multiple plastid DNA sequences, Clarkson *et al.* (2004) speculate that *N. rustica* may be more closely related to *N. knightiana* than to *N. paniculata*. It is also evident from their work, as well as the work of others (Akoi and Ito, 2000), that the most recent common ancestor of the *N. paniculata*/*N. knightiana* pair may actually be the maternal donor of *N. rustica* since *N. rustica* was shown to diverge prior to the diploid species pair.

N. rustica is a diverse species with high levels of phenotypic variation (Goodspeed, 1954). Several researchers, such as Comes (1899), have historically worked to classify accessions into intraspecific varieties in order to manage some of the diversity they were observing. While Danert (1963) recognized six varieties, Goodspeed (1954) only included three in his work, although he noted that they were included only as a means of representing the extremes among a continuum of variation. The brasilia variety is immediately recognizable by its puckered ovate leaves and its thick ridged stem. The pumila type strain, as described by Goodspeed (1954), is the shortest variety type (60-80 cm) with many stems coming from the ground. It is also characterized by elliptic leaves and a panicle type inflorescence with many peduncles, giving it a brushy appearance. The only variety believed to have not been a cultigen is known as pavonii. This variety has a prominent main axis with branches from each node and a loose panicle inflorescence characterized by elliptic capsules which are dehiscent.

Other evidence for *N. rustica* diversity comes from the intraspecific breeding and selection experiments of Smith and Bacon (1941). They bred different varieties of *N. rustica* and selected for increased height, leaf number and largest leaf area. Using different crossing methods, they were able to determine that the transgressive segregants were caused by the presence of different combinations of alleles affecting these traits in each variety. Also, individual accessions with unique characteristics, such as anthocyanin in the ovaries, aurea coloration, and white seed coats, point to a diverse genetic base (personal observations). *N. rustica* accessions additionally show varying levels of characteristics derived from the progenitor species, such as in type and distribution of trichomes (Goodspeed, 1954), level of leaf surface puckering or presence of ridged stems (personal observations). These varying combinations of traits could be the result of considerable chromosomal rearrangement since the time of divergence, as was demonstrated to have occurred in the 18-5.8-26S rDNA locus by Matyasek *et al.* (2003).

Molecular markers, such as randomly amplified polymorphic DNA (RAPDs), restriction or amplified fragment length polymorphisms (RFLPs or AFLPs), and simple sequence repeats (SSRs), have become useful tools in the assessment of the genetic basis of variation. Previously, estimations of genetic diversity had to be extrapolated from phenotypic data analyses such as Euclidean distances and principle component analysis. Molecular markers have been used to assess the genetic diversity within the germplasm bases of agricultural crops, such as wheat (Al Khanjari *et al.*, 2007), cotton (Lacape *et al.*, 2007) and soybean (Maughan *et al.*, 1996), as well as in horticultural crops, including coffee (Moncada and McCouch, 2004), radish (Rabbani *et al.*, 1998), Italian pepper (Portis *et al.*, 2006) and plantains (Crouch *et al.*, 2000). Even more recently, as the prices for sequencing have decreased, direct sequence differences among individuals have been used to determine ancestral relationships among species (Clarkson *et al.*, 2004) and levels of population variation within species (Jordan *et al.*, 1996).

With the exception of *N. tabacum*, little work has been done to quantify intraspecific variation within *Nicotiana* species. The genus is large, with 76 currently recognized naturally occurring species (Knapp *et al.*, 2007), and has been well characterized (most notably by Goodspeed, 1954). While the majority of species occur from western South America through the southwestern United States, a large number have been discovered in Australia, and there are a few reports of species isolated from other remote areas, such as *N. africana*.

Analyses of the phylogenetic relationships among species using RAPDs (Yu and Lin, 1997), AFLPs (Ren and Timko, 2001), SSRs (Hyunsook Moon, personal communications), ribosomal DNA sequences (Chase *et al.*, 2003) and plastid sequences (Clarkson *et al.*, 2004) have led to little change from the early taxonomic work that was based on morphology and cytogenetics. Nearly 40% of the species within the genus are allopolyploids, many of which have shown considerable amounts of genome rearrangement since speciation (Leitch *et al.*, 2007; Lim *et al.*, 2007). However, upon AFLP analysis of 46 diverse accessions of *N. tabacum*, Ren and Timko (2001) reported what they considered to be a "relatively small" level of genetic polymorphism compared to the observed phenotypic variation among the tobacco accessions.

The purpose of this investigation was to perform the first assessment of the molecular and phenotypic variation present within *N. rustica*. The *trnL-F* chloroplast intergenic region, published recently by Clarkson *et al.* (2004), was chosen from a set of sequences to represent a maternally inherited locus. This locus, in combination with a genome wide AFLP profile, was studied for the potential to reveal the ancestral and more recent relationships among the accessions investigated. Morphological data was analyzed by principle component analysis to look for specific clustering patterns and the distances between accessions were determined. Additionally, the correlation between estimates of variability based on the phenotypic data and on the genetic data was assessed.

MATERIALS AND METHODS

Plant material and DNA isolation

The material examined in this study is comprised of the 86 accessions of the *Nicotiana rustica* Collection within the U.S. *Nicotiana* Germplasm Collection. A summary of this material is provided in Table 2.1. Plant material was grown in sterile 50-50 peat-sand mixture in a growth chamber at 25°C with a 16 h light cycle. A single young leaf per plant of each accession was chosen for DNA isolation. DNA was extracted with Plant DNAzol

Table 2.1 *N. rustica* Accession Identifiers and Origins.

Collection Identifier	Plant Introduction Number	Alternative Identifiers	Origin
TR1	499162	4384-HHS	Experimental
TR2	499163	4385 L-5-6	Experimental
TR3	499164	4386 L-5-6	Experimental
TR4	499165	4390 L-5-2-1	Experimental
TR5	499166	4398 L-5-2-1	Experimental
TR6	499167	4399 L-5-2-1	Experimental
TR7	499168	43054 L-5-2-1	Experimental
TR8	499169	43101 L-2-1B × L-6-2-1	Experimental
TR9	499170	43102-1 L-6-2-1 × L-5-2-1B	Experimental
TR10	499171	43103-5 L-5-2-1 × L-6-2-1	Experimental
TR11	499172	43104-1 L-5-2-1B × L-6-2-1	Experimental
TR12	499173	4401 L-5-2-1B	Experimental
TR13	499174	Brasilia #7	Unknown
TR14	499175	Brasilia #23	Unknown
TR15	34753	Brasilia Selvaggio; *N. rustica* var. *brasilia* Schrank	Brazil
TR16	34752	Brasilia Leccese	Brazil
TR17	34754	Erbasanta	Brazil
TR18	499176	68 Olson	
TR20	499178	German #2	Unknown
TR21	499179	German #1	Unknown
TR22	499180	Mahorka #1 AC 18/7	Russia
TR23	499181	Mahorka #2 Armenia	Russia
TR24	499182	Mahorka #3 Voronezhskaia	Russia
TR25	499183	Mahorka #4 Tall Green	Russia
TR26	499184	Mahorka #5	Russia
TR27	499185	Mahorka #6 Yellow #109	Russia
TR28	499186	Mahorka #7 Pekhletzenskaia	Russia
TR29	499187	Mahorka #8 Prosechenskaia Local	Russia
TR30	499188	Mahorka #9 Stapukhenskaia	Russia
TR31	499189	Mahorka #10 Sorotooskaia	Russia
TR32	499190	Mahorka #11 Stalingradskaia	Russia
TR33	499191	Mahorka #12 Ivisenskaia Local	Russia
TR34	499192	Kostoff White Seed #14	Russia
TR35	499193	BAK #46	Russia
TR36	499194	Koriotes Dark-blue	Russia
TR37	499195	Jainkaya Soldata	Russia
TR38	499196	Jainkaya Black-blue #54	Russia
TR39	499197	Drosgi Black-blue #45	Russia
TR40	499198	14 NO. 23057	Russia
TR41	499199	Edinburg #25	Russia
TR42	499200	JA. BOT. CAR. #30	France
TR43	499201	R. BOT. CAR. #29	Lithuania
TR44	499202	Harbin #6	China

Table 1 Continued.

TR45	269933	Normal	Pakistan
TR46	269934	Matsuj Field	Pakistan
TR47	269935	Buni Field	Pakistan
TR48	269936	Dumont	Pakistan
TR49	499203	Chinensis	Turkey
TR50	499204	Campanulata	New Zealand
TR51	499205	Acutiflora	New Zealand
TR52	499206	Fructicora	New Zealand
TR53	499207	Acutifolia	New Zealand
TR54	240355	Nordugel	New Guinea
TR55	478886	GC-1	India
TR56	481453	Hasankeyf	Turkey
TR57	475761	PNE 241-5	Pakistan
TR58	475762	PNE 362-4	Pakistan
TR59	475763	PNE 369-3	Pakistan
TR60	475764	PNE 373-13	Pakistan
TR61	475765	PNE 407-5	Pakistan
TR62	475766	PNE 412-8	Pakistan
TR63	475767	PNE 417-4	Pakistan
TR64	475768	PNE 418-6	Pakistan
TR65	475769	PNE 420-6	Pakistan
TR66	475770	PNE 427-4	Pakistan
TR67	494878	ZFA 3544	Zambia
TR68	494879	ZFA 3561	Zambia
TR69	494880	ZFA 3564	Zambia
TR70	500927		Zambia
TR71	500931		Zambia
TR72	500932		Zambia
TR73	500933		Zambia
TR74	500936		Zambia
TR75	505664	ZM/A5133	Zambia
TR76	481861	Maras	Turkey
TR77	481867	Karabaglar	Turkey
TR78		*selection from TR36	Russia
TR79		*selection from TR42	France
TR80		*selection from TR52	New Zealand
TR81			Unknown
TR82	555556		Unknown
TW116	243561	*N. rustica* var. *pavonii*, 44	Peru
TW117	555554	*N. rustica* var. *brasilia*, 48	Unknown
TW118	555555	*N. rustica* var. *pumila*, 49	Unknown
TW119	555692	*N. rustica*, 49A	Unknown
TW120	555693	*N. rustica*, 49B	Unknown
TW100	241769	*N. paniculata*; 40A	Unknown
TW145	555574	*N. undulata*; 61A	Unknown

(Invitrogen) according to the manufacturer's instructions with slight modifications to allow for greater amounts of tissue. Additionally, tissue was ground in Lysing Matrix A tubes (MP Bioscience) in the presence of DNAzol and ribonuclease A (Sigma) in a FP120 FastPrep for two cycles of 20 seconds each. After resuspension in TE buffer (10 mM Tris · HCl pH 8.0, 1 mM EDTA pH 8.0, 1 M NaCl), the DNA was quantified in a Hoefer DyNA Quant 200 flourometer according to product specifications.

Table 2.2 AFLP Pre-amplification and Selective Amplification Primers.

Primer Combination	E-Primer	M-Primer
Pre-amp.	GACTGCGTACCAATT	GATGAGTCCTGAGTA
1	- AAC	- CAC
2	- AAC	- CAG
3	- AAC	- CTG
4	- AAG	- CTA
5	- AAG	- CTT
6	- ACA	- CTG
7	- ACA	- CTT
8	- ACC	- CAC
9	- ACC	- CAG
10	- ACC	- CAT
11	- ACC	- CTC
12	- ACC	- CCT
13	- ACC	- CGA
14	- ACC	- CGC
15	- ACC	- CGG
16	- ACG	- CAT
17	- ACG	- CTA

AFLP Data

AFLP analysis, using *EcoRI* and *MseI* restriction enzymes, was conducted as published previously by Vos *et al.* (1995), with modifications by Myburg *et al.* (2001) and by Moon and Nicholson (2007). Selective amplification products were run on 8% polyacrylamide gels in LI-COR 4300 DNA Analyzers (LI-COR Biosciences). Banding patterns were analyzed visually and polymorphic loci were scored by hand for presence or absence. Seventy-eight polymorphic loci, ranging from 50 to 650 bp, generated from 17 primer combinations were chosen for analysis based on the clarity of their polymorphisms in *N. rustica*. A list of the pre-amplification and selective amplification primers used is provided in Table 2.2.

Sequence Data

Primers were designed for polymerase chain reaction (PCR) amplification of the chloroplast *trnL-F* DNA locus for *N. rustica* (GenBank accession AJ577439) (Forward: AACAAAAATGGGCAATCCTG, Reverse: TTTTATCAAAGCCGGGGAAT, 748 bp expected product). Amplification reactions were carried out in an Eppendorf Mastercycler (Eppendorf) in 50 μL volumes containing approximately 125 ng of template DNA, 1 X ThermoPol Buffer (10 mM KCl, 10 mM $(NH_4)_2SO_4$, 20 mM Tris-HCl, 2 mM $MgSO_4$, 0.1% Triton ×-100; New England Biolabs), 25 μM of each primer, 10 mM dNTPs and 2U of *Taq* DNA polymerase (New England Biolabs) with the following cycling program: 4 min at 95°C, followed by 35 cycles of 45 sec at 95°C, 45 sec at 63°C, 1 min at 72°C, with a final extension of 5 min at 72°C. The PCR products were cleaned using a QIAquick PCR Purification Kit (QIAgen) according to product specifications and then quantified as described previously. Sequencing reactions were performed by Northwoods DNA Inc. (Bemidji, Minnesota).

Phenotypic data

In the summer of 2005, the 86 *N. rustica* accessions were planted in 12 plant plots in a randomized complete block design (RCBD) with two replications at the Oxford Tobacco Research Station in Oxford, North Carolina. Plants were transplanted and cultivated according to the standard recommendations for flue-cured tobacco (Smith *et al.*, 2006; Smith and Wood, 2006). Four weeks after transplanting, data taking began on days to flower and continued once a week thereafter for seven weeks. Approximate days to flower per plot was determined upon the first reading in which at least 50% of the plants within the plot were flowering. Data on other morphological characters was taken ten weeks after transplanting on each interior plant within a plot, including stem type (ridged or smooth), leaf surface type (smooth, slightly or highly puckered), leaf shape (standard botanical classification), plant height including inflorescence, middle leaf length and width, flower length, corolla width and seed capsule diameter. Additionally at this time, five interior plants of each plot were topped (inflorescences removed). Two weeks later a leaf from approximately midways up the stalk (subsequently referred to as "middle leaf") was harvested from each of these plants. Leaves from a single plot were pooled into a single mesh bag and allowed to air cure. The dried samples were ground and the percent total alkaloids (PTA) were determined by the methods of Davis (1976) and Harvey *et al.* (1969).

The same experiment was planted in 2006 with two replications in each of two locations, the Oxford Tobacco Research Station and the Central Crops Research Station (Clayton, NC). Ten weeks after transplanting, additional measurements of plant height and middle leaf length and width were taken for plots at the Oxford station. In both locations, a middle leaf was removed from five plants within each plot before topping. Samples were prepared and analyzed as in 2005 to determine pre-topping alkaloid levels. As done in 2005, plots were topped and two weeks later a middle leaf was again removed from each of the five interior plants for each plot to determine the PTA after topping. Accessions in this season were also evaluated for variety type according to the descriptions provided by Goodspeed (1954). Each accession was noted as being either brasilia, pumila, pavonii, or an intermediate type.

Data analysis

Genetic Data

Sequences were edited with Finch TV version 1.4.0 (Geospiza Inc.) and aligned using ClustalW (Thompson *et al.*, 1994) within SNAP Workbench (Price and Carbone, 2005; Aylor *et al.*, 2006). Due to lack of variation within *N. rustica*, sequence data was excluded from further analyses. The binary AFLP profiles were collapsed into haplotypes using SNAP Map (Aylor *et al.*, 2006). SNAP Clade and Matrix (Bowden *et al.*, 2008) were used to determine site compatibility and linkage disequilibrium of the AFLP loci. Alternative parsimony analysis was performed within SNAP Workbench using PAUP (Swofford, 1998).

Additional analyses of the AFLP data were conducted using NTSYSpc ver. 2.2 (Rolfe, 2007). Genetic similarities among accessions were calculated using Jaccard's coefficient (Jaccard, 1908), simple matching (Sokal and Sneath, 1963), and Dice's coefficient (Dice, 1945) and dissimilarities were calculated with Nei's coefficient (Nei, 1972). Phylograms were generated from each of these matrices and the cophenetic correlation coefficient of each was determined. The analysis revealed that Jaccard's coefficient had the highest correlation value ($r = 0.71$). UPGMA (unweighted pair-group method with arithmetic averages) clustering was performed on this similarity matrix within the SAHN (sequential agglomerative hierarchical nested clustering; Sneath and Sokal, 1973) program of NTSYSpc and produced 12 equal trees, from which a consensus tree was constructed. Additionally, Jaccard genetic distances between accessions, based on their AFLP profiles, were calculated in SAS v. 9.1.3 (SAS Institute Inc.) and imported into NTSYSpc where clustering was performed by the neighbor-joining method (Saitou and Nei, 1987). A single phylogeny was produced which had a cophenetic value correlation of 0.51.

Due to the independent nature of AFLP loci (see Figure 2.2 B), the data set was also analyzed with STRUCTURE 2.2 (Pritchard *et al.*, 2000; Falush *et al.*, 2003 and 2007) to determine possible population structure. Data was coded according to the suggestions for dominant markers provided by the distributors. Lambda was determined by running one simulation assuming no admixture, correlated allele frequencies and one population (K = 1). Since it is known that accessions of *N. rustica* have been frequently crossed in breeding programs, all further simulations were performed assuming population admixture. Additional parameters included correlated allele frequencies and the estimated value of lambda, 0.4413. All simulations were run with a 50,000 cycle burn-in followed by 100,000 MCMC iterations.

Values of K examined ranged from 2 to 6. Accessions were assigned to the population for which they had greater than 0.5 probability of membership.

Phenotypic Data

An analysis of variance (ANOVA) was performed for each of the quantitative morphological characters in SAS v. 9.1.3. The common main effects for each analysis were entry (accession) and replication. Year was included during analyses of height, middle leaf length and width and post-topping PTA and location was an additional variable in PTA analyses. Phenotypic data was additionally analyzed by principle component analysis in SAS where quantitative variables were entered as the entry (accession) means. Average days-survival index for black shank race 1 was also included as a quantitative variable (refer to Chapter 3) since disease resistance properties are also measurable phenotypes. Inclusion of all of the quantitative variables, except for pre-topping PTA, resulted in the greatest explanation of variation within the first two components, 59%. Principle component 2 was plotted against principle component 1 and the observations were identified by each of the six categorical variables, including origin, proposed variety types and STRUCTURE groupings, in an attempt to characterize the observed grouping of the observations along the two principle axis. Pair wise distances between accessions were calculated by the DGOWER method (a dissimilarity measure) in PROC DISTANCE (SAS) using all of the quantitative variables and three of the categorical variables (leaf surface, leaf shape and stem type). A geometrical representation of these distances in a plane was obtained through non metric dimensional analysis (PROC MDS, SAS).

Correlation Analysis

Including the STRUCTURE groupings as a categorical variable during the principle component analysis provided a glimpse of how well the genetic populations determined by STRUCTURE correlated with the distribution of individuals based on morphological data. Additional correlation analyses were carried out in R (www.r-project.org). The Bray-Curtis dissimilarities were calculated from the AFLP profiles of the accessions in R (distance, vegan) and later used for Kruskal's non metric dimensional scaling (NMDS) analysis (isoMDS, MASS). NMDS allows for the representation of the accessions in a plane (dimension = 2) while preserving the distance (dissimilarity) between them.

The quantitative morphological variables were examined for their power to predict the AFLP NMDS configuration (two-dimension coefficients) with a GAM (generalized additive models with integrated smoothness estimation) analysis in R (surf, labdsv). Based on this, the variables corolla width, flower length, days to flower, post-topping alkaloid level and average days-survival index for black shank race 1 were chosen to recreate a morphological characterization of the accessions using NMDS on their matrix of Euclidean distances. Procrustes analysis was run in R (protest, vegan library) with ten thousand permutations to perform a pairwise comparison of the genetic and morphological distances. The correlation of a symmetric Procrustes rotation of these two configurations was determined to be 0.2101 (p = 0.0427). The inclusion of all of the morphological variables led to a much lower correlation.

RESULTS

Genetic data analysis

An alignment of the sequence data revealed that the *trnL-F* region showed no variation among the *N. rustica* accessions. The *N. rustica* sequence was identical to the sequence obtained for *N. paniculata* and both were indistinguishable from three sequences published by Clarkson *et al.* (2004; GenBank accessions AJ577439, AJ577398 and AJ577417; *N. rustica*, *N. paniculata* and *N. knightiana*, respectively). The *N. undulata* sequence was almost identical to the previously published sequence (GenBank accession AJ577419) except for the insertion of a guanine at site 551 in the new sequence (see Figure 2.1). While there were considerable differences between the *N. undulata* sequences and the sequences of the other species, this data had to be excluded from further analyses due to the lack of intraspecific variation among the *N. rustica* accessions.

Figure 2.1 Sequence Alignment. A sequence alignment for *trnL-F*, as performed by ClustalW, of published *N. paniculata* (AJ577398), *N. rustica* (AJ577439) and *N. undulata* (AJ577419) sequences along with a representative *N. rustica* (TR15) sequence and the *N. undulata* (TW145) sequence obtained by this study. The stars below the sequences indicate nucleotides which are identical in all sequences.

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AJ577398        GCAATCCTGAGCCAAATCCTGTTTTCCGAAAACAAACAAAGGTTCAGAAAAAAAAAGGATA
AJ577439        GCAATCCTGAGCCAAATCCTGTTTTCCGAAAACAAACAAAGGTTCAGAAAAAAAAAGGATA
TR15            ---------------ATCCTGTTTTCCGAAAACAAACAAAGGTTCAGAAAAAAAAAGGATA
AJ577419        GCAATCCTGAGCCAAATCCTGTTTTCCGAAAACAAACAAAGGTTCAGAAAAAAAAAGGATA
TW145           ---------------ATCCTGTTTTCCGAAAACAAACAAAGGTTCAGAAAAAAAAAGGATA
                               *********************************************

AJ577398        GGTGCAGAGACTCAATGGAAGCTATTCTAACAAATGGAGTTAAATGCGTTGGTAGAGGAA
AJ577439        GGTGCAGAGACTCAATGGAAGCTATTCTAACAAATGGAGTTAAATGCGTTGGTAGAGGAA
TR15            GGTGCAGAGACTCAATGGAAGCTATTCTAACAAATGGAGTTAAATGCGTTGGTAGAGGAA
AJ577419        GGTGCAGAGACTCAATGGAAGCTATTCTAACAAATGGAGTTAAATGCGTTGGTAGAGGAA
TW145           GGTGCAGAGACTCAATGGAAGCTATTCTAACAAATGGAGTTAAATGCGTTGGTAGAGGAA
                ************************************************************

AJ577398        TCTTTACATCGAAACTTCAGAAAGAAAAAGAATGAAGTGAAGGATAAACGTATATACATA
AJ577439        TCTTTACATCGAAACTTCAGAAAGAAAAAGAATGAAGTGAAGGATAAACGTATATACATA
TR15            TCTTTACATCGAAACTTCAGAAAGAAAAAGAATGAAGTGAAGGATAAACGTATATACATA
AJ577419        TCTTTACATCGAAACTTCAGAAAGAAAAAGAATGAAGTGAAGGATAAACGTATATACATA
TW145           TCTTTACATCGAAACTTCAGAAAGAAAAAGAATGAAGTGAAGGATAAACGTATATACATA
                ************************************************************

AJ577398        CGTATTGAATACTATATCAAATGATTAATGATGACCCGAATCCATATTTTTTCTATAAAA
AJ577439        CGTATTGAATACTATATCAAATGATTAATGATGACCCGAATCCATATTTTTTCTATAAAA
TR15            CGTATTGAATACTATATCAAATGATTAATGATGACCCGAATCCATATTTTTTCTATAAAA
AJ577419        CGTATTGAATACTATATCAAATGATTAATGATGACCCGAATCCGTATTTTTTCTATAAAA
TW145           CGTATTGAATACTATATCAAATGATTAATGATGACCCGAATCCGTATTTTTTCTATAAAA
                ******************************************* ****************

AJ577398        AATAGAAGAGTTGGTGTGAATCGATTCTACATTGAAGAAAGAATCGAATATTCATTGATC
AJ577439        AATAGAAGAGTTGGTGTGAATCGATTCTACATTGAAGAAAGAATCGAATATTCATTGATC
TR15            AATAGAAGAGTTGGTGTGAATCGATTCTACATTGAAGAAAGAATCGAATATTCATTGATC
AJ577419        AATGGAAGAGTTGGTGTGAATCGATTCTACATTGAAGAAAGAATCGAATATTCATTGATC
TW145           AATGGAAGAGTTGGTGTGAATCGATTCTACATTGAAGAAAGAATCGAATATTCATTGATC
                *** ********************************************************

AJ577398        AAATCATTCACTCCATAGTCTGACAGATCTTTTGAAGAACTGATTAATCGGACGAGAATA
AJ577439        AAATCATTCACTCCATAGTCTGACAGATCTTTTGAAGAACTGATTAATCGGACGAGAATA
TR15            AAATCATTCACTCCATAGTCTGACAGATCTTTTGAAGAACTGATTAATCGGACGAGAATA
AJ577419        AAATCATTCACTCCATAGTCTGATAGATCTTTTGAAGAACTGATTAATCGGACGAGAATA
TW145           AAATCATTCACTCCATAGTCTGATAGATCTTTTGAAGAACTGATTAATCGGACGAGAATA
                *********************** ************************************

AJ577398        AAGATAGAGTCCCGTTCTACATGTCAATACCGGCAACAATGAAATTTATAGTAAGAGGAA
AJ577439        AAGATAGAGTCCCGTTCTACATGTCAATACCGGCAACAATGAAATTTATAGTAAGAGGAA
TR15            AAGATAGAGTCCCGTTCTACATGTCAATACCGGCAACAATGAAATTTATAGTAAGAGGAA
AJ577419        AAGATAGAGTCCCGTTCTACATGTCAATACCGGCAACAATGAAATTTATAGTAAGAGGAA
TW145           AAGATAGAGTCCCGTTCTACATGTCAATACCGGCAACAATGAAATTTATAGTAAGAGGAA
                ************************************************************

AJ577398        AATCCGTCGACTTTAAAAATCGTGAGGGTTCAAGTCCCTCTATCCCCAAAAAGACTATTT
AJ577439        AATCCGTCGACTTTAAAAATCGTGAGGGTTCAAGTCCCTCTATCCCCAAAAAGACTATTT
TR15            AATCCGTCGACTTTAAAAATCGTGAGGGTTCAAGTCCCTCTATCCCCAAAAAGACTATTT
AJ577419        AATCCGTCGACTTTAAAAATCGTGAGGGTTCAAGTCCCTCTATCCCCAAAAAGACTATTT
TW145           AATCCGTCGACTTTAAAAATCGTGAGGGTTCAAGTCCCTCTATCCCCAAAAAGACTATTT
                ************************************************************

AJ577398        CACTCCC----------TCCGACCCCCTTTCCTTAGCGGTTCCAAATTCCTTATCTTTCT
AJ577439        CACTCCC----------TCCGACCCCCTTTCCTTAGCGGTTCCAAATTCCTTATCTTTCT
TR15            CACTCCC----------TCCGACCCCCTTTCCTTAGCGGTTCCAAATTCCTTATCTTTCT
AJ577419        CACTCCCCAACTATTTATCCGACCCCCTTTCCTTAGCGGTTCCAAATTCCTTATCTTTCT
TW145           CACTCCCCAACTATTTATCCGACCCCCTTTCCTTAGCGGTTCCAAATTCCTTATCTTTCT
                *******          *******************************************

AJ577398        CATTCACTCTATTCTTTTAGAAATGGATTTGAGCGTAAATGGCTTTCTCTTATCACAAGT
AJ577439        CATTCACTCTATTCTTTTAGAAATGGATTTGAGCGTAAATGGCTTTCTCTTATCACAAGT
TR15            CATTCACTCTATTCTTTTAGAAATGGATTTGAGCGTAAATGGCTTTCTCTTATCACAAGT
AJ577419        CATTCACTCTATTCTTTTAGAAATG-ATTTGAGCGTAAATGGCTTTCTCTTATCACAAGT
TW145           CATTCACTCTATTCTTTTAGAAATGGATTTGAGCGTAAATGGCTTTCTCTTATCACAAGT
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AJ577398        CTTGTGATATATATGATACACATAGAAATAAACATCTTTGAGCAAGGAATCCCTAGTTGA
AJ577439        CTTGTGATATATATGATACACATAGAAATAAACATCTTTGAGCAAGGAATCCCTAGTTGA
TR15            CTTGTGATATATATGATACACATAGAAATAAACATCTTTGAGCAAGGAATCCCTAGTTGA
AJ577419        CTTGTGATATATATGATACACATAGAAATGAACATCTTTGAGCAAGGAATCCCTAGTTGA
TW145           CTTGTGATATATATGATACACATAGAAATGAACATCTTTGAGCAAGGAATCCCTAGTTGA
                ***************************** ******************************

AJ577398        ATGATTCCCTATCAATATCATTACTCATACTGAAACTTACAAAGTCATCTTTTTGAAGAT
AJ577439        ATGATTCCCTATCAATATCATTACTCATACTGAAACTTACAAAGTCATCTTTTTGAAGAT
TR15            ATGATTCCCTATCAATATCATTACTCATACTGAAACTTACAAAGTCATCTTTTTGAAGAT
AJ577419        ATGATTCCCTATCAATATCATTACTCATACTGAAACTTACAAAGTCATCTTTTTGAAGAT
TW145           ATGATTCCCTATCAATATCATTACTCATACTGAAACTTACAAAGTCATCTTTTTGAA---
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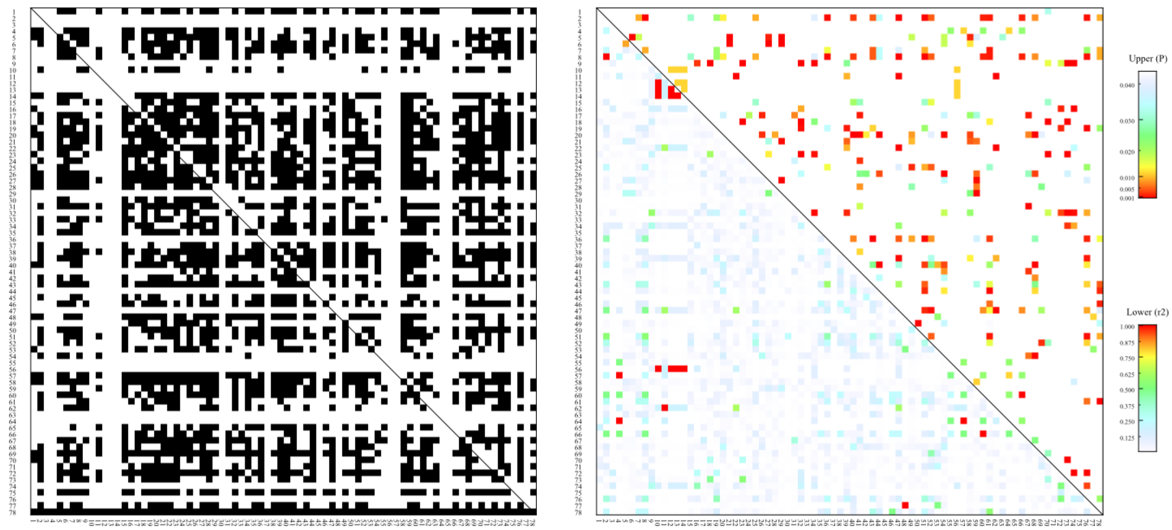


A **B**

Figure 2.2 AFLP Loci Compatibility and Linkage. (A) Site compatibility matrix generated by SNAP Clade and Matrix. Each black square indicates incompatibility between two loci. (B) Linkage and correlation matrix where the sites are seen to be unlinked in the lower half and generally uncorrelated in the upper half.

Where as Ren and Timko (2001), working with accessions of *N. tabacum*, saw an average of 23.8 polymorphic loci per AFLP selective amplification primer combination, only an average of 4.6 reliable polymorphic loci per primer combination were obtained from AFLP analysis of *N. rustica*. Collapsing the AFLP data for the 86 *N. rustica* accessions resulted in 85 different haplotypes. While TR 16 and TR 70 had identical profiles based on the available data, all other *N. rustica* accessions examined were unique genotypes. However, almost all of the informative sites were incompatible with each other (Figure 2.2 A). This was also evident in the failure of the PAUP analysis to go to completion, even after limiting the number of possible "trees" to 2000. A strict tree was generated but was little more than a polytomy, retaining no information about the ancestral relationships among individuals. Investigation of the linkage disequilibrium revealed that all loci were generally uncorrelated and unlinked (Figure 2.2 B), which met the assumption required for analysis with STRUCTURE.

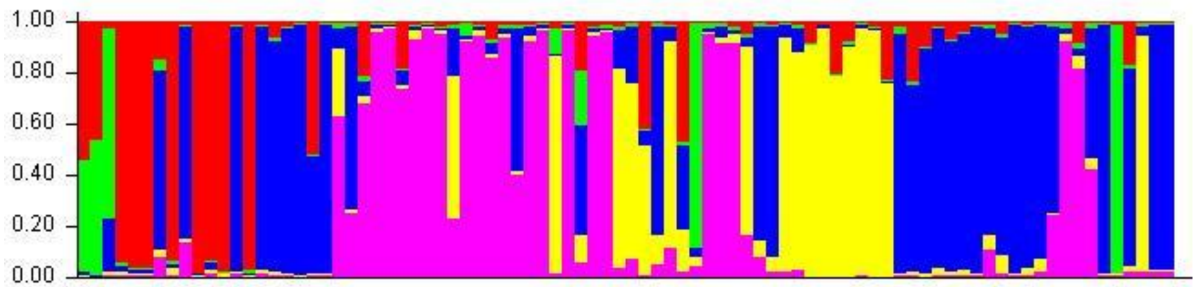
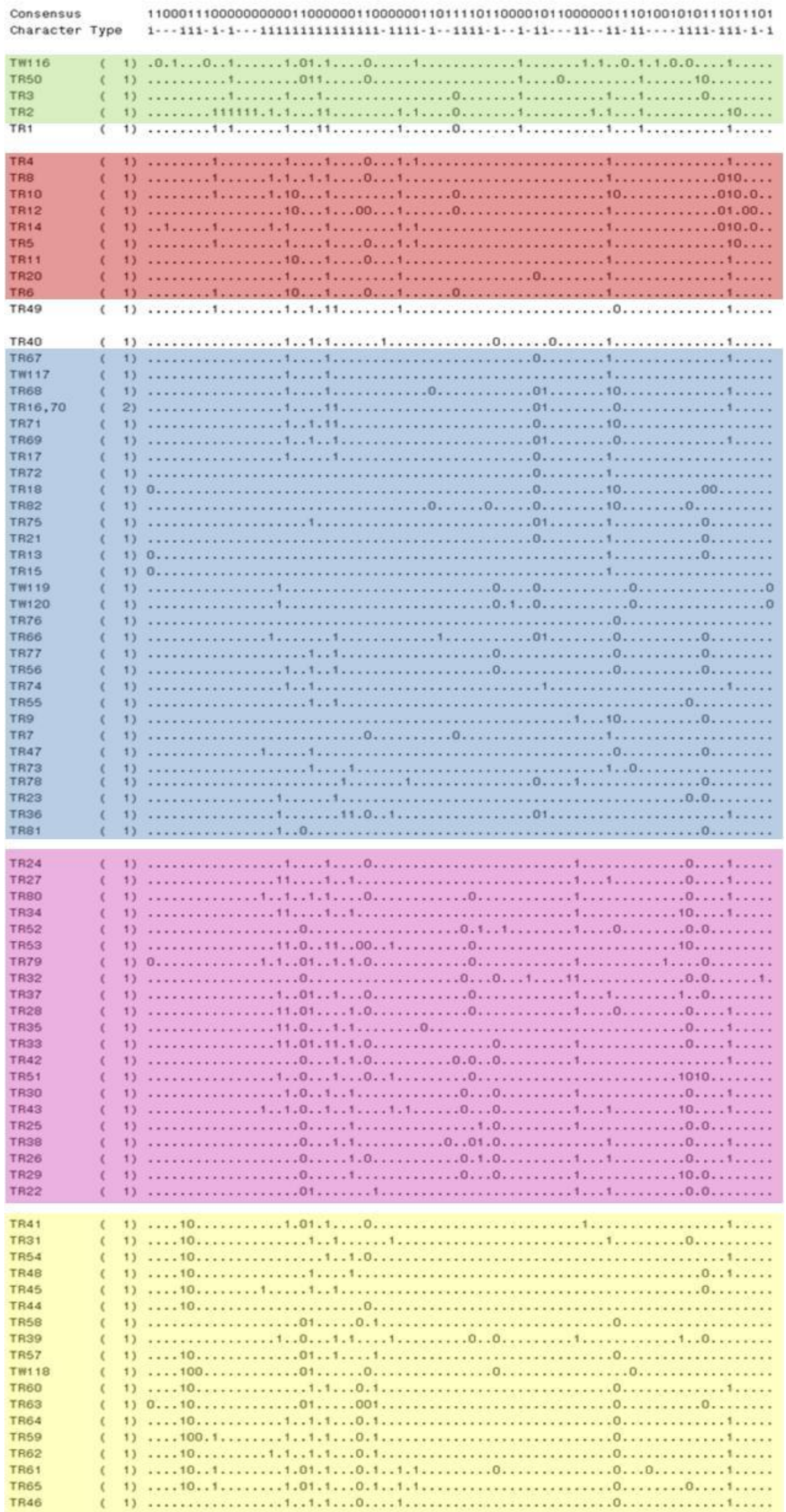


Figure 2.3 Structure Output. Accessions are arranged from left to right along the x-axis in the same order as they appear in Table 1, with the exception of the sequence: …, TR 37, TR 38, TR 40, TR 41, TR 39, TR 42, TR 43, … The y-axis indicates the probability of population membership and each of the five populations is represented by a different color.

Simulations were run in STRUCTURE assuming values of K from 2 to 6. Although 6 populations had the best overall mean value of the natural log of the likelihood (-1616.6; estimated Ln P|data = -2433.0), it did not improve much upon 5 populations (-1665.1) which had a better probability given the data (estimated Ln P|data = -2062.2). Thus, five non-independent populations were assumed to exist. The graphical output of the probability of accessions belonging to each population is presented in Figure 2.3, where each column represents an accession, each color represents one of the five populations, and the length of the colored bar indicates the probability of that accession belonging to that population. For example, TR 2 (the second column from the left) has an almost equally likely probability of belonging to either the red or green populations, but is obviously not related to members of the purple, yellow or blue populations. When the haplotype map was arranged to reflect the STRUCTURE output (Figure 2.4), clear patterns of allelic variation between populations were revealed (see also Appendix C). Most accessions clearly fall into one population or another, however TR 40 and TR 49 do not (<0.5 probability of belonging to any group).

TR 1 through TR 12, donated by H. H. Smith from what is believed to have been a breeding and selection program for studying quantitative variation, all share a high probability of belonging to the same group (represented in Figure 2.3 by the color red), with the exception of TR 3 (P_{red} = 0.024), TR 7 (P_{red} = 0.143) and TR 9 (P_{red} = 0.009). Excluding TR 47 (P_{yellow} = 0.120) and TR 66 (P_{yellow} = 0.011), the yellow group contains all of the accessions from

Figure 2.4 Haplotype Map. Map of the *N. rustica* haplotypes where the loci are coded "1" for presence, "0" for absences and "." for matches to the consensus sequence. The accessions are arranged according to STRUCTURE population probabilities and are shaded to match the output in Figure 2.3.



Pakistan. The purple group contains the four accessions from New Zealand and most of the accessions from Russia. Four other accessions from Russia retain approximately 0.25 probability of belonging to this group and the remaining two, TR 40 (P_{purple} = 0.059) and TR 41 (P_{purple} = 0.018), have only a nominal probability of membership to this group. The largest group (blue) contains the three oldest accessions in the collection which are from Brazil, all the accessions from Zambia, and all but one accession from Turkey (TR 49, P_{blue} = 0.328).

The SAHN consensus tree generated from the Jaccard similarities among the accessions is presented in Figure 2.6. STRUCTURE groupings are indicated on the ends of the branches and a strong correlation can be seen between the results of these two analyses. All of the accessions from Pakistan form a monophyletic group which is sister to the rest of the yellow population, although TR 46 is not included within this clade. Additionally, all of the members of the purple group are contained within a single clade, with the exception of TR 22. Other than the accessions from Pakistan, there is no definitive correlation between origin and genetic similarity based on this analysis, nor is there a correlation with variety type.

Pair-wise genetic distances (Jaccard's) ranged from zero (between TR 16 and TR 70) to 0.547 (between TR 2 and TR 63), with an average of 0.281. The actual values may be inflated, however, due to the exclusion of nopolymorphic loci during analysis. Figure 2.7 depicts the neighbor-joining tree generated from these distances and there is a fair degree of agreement with the consensus tree generated by SAHN (Figure 2.6). In the neighbor-joining tree, the members of the yellow STRUCTURE population appear in a single clade, which additionally includes TR 55 (blue group). Although the pairings are similar, the accessions from Pakistan are no longer monophyletic. The purple group has been divided into two clades, where TR 22, TR 25, TR 29, TR 32 and TR 52 appear as a clade within the region of the tree dominated by the blue group. The red population is much more defined in this analysis than in the SAHN analysis, except TR 1 is not included within this clade. According to STRUCTURE, however, TR 1 had almost equal probabilities of belonging to either the green or red populations.

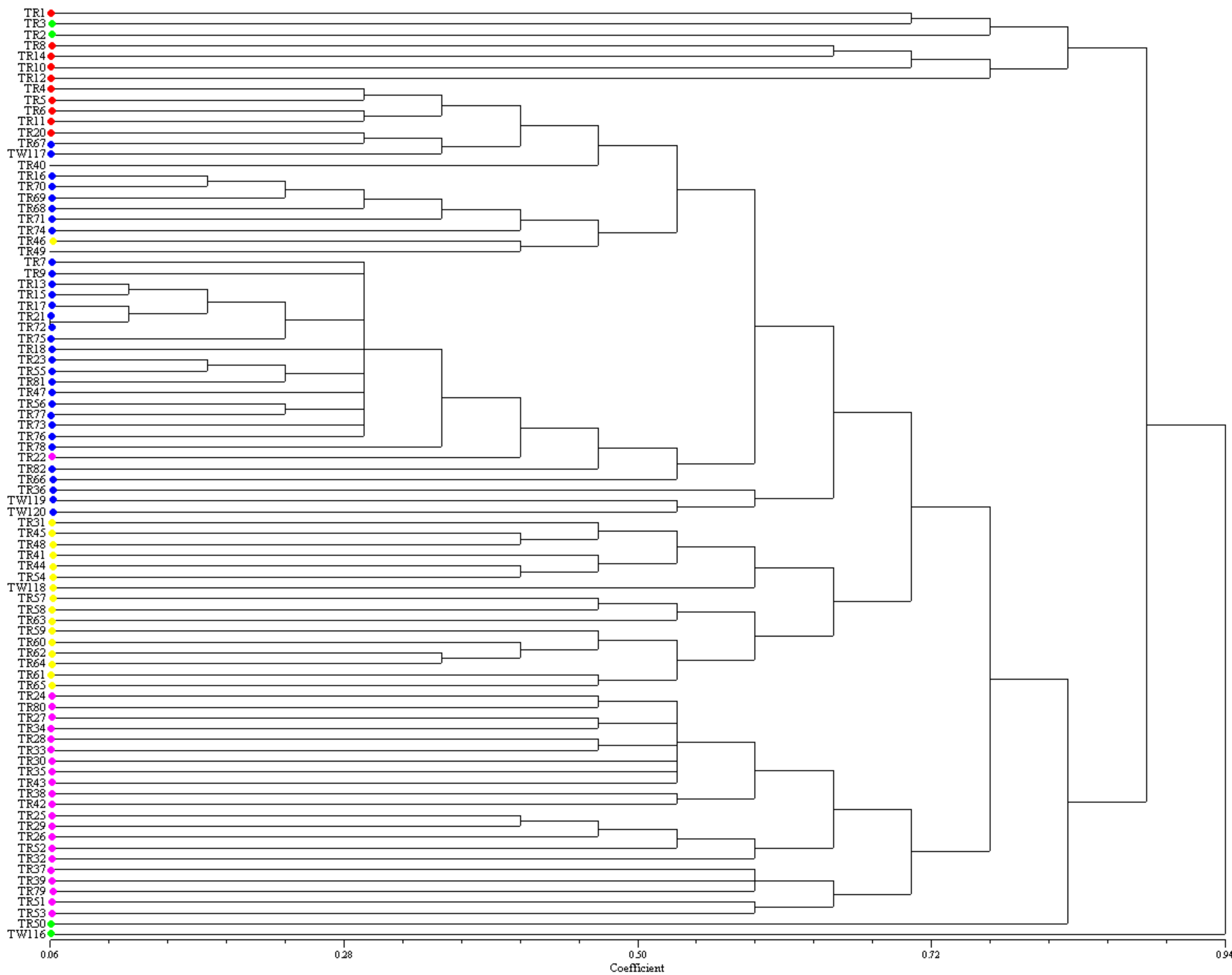


Figure 2.5 UPGMA-SAHN Consensus Tree. Dots located at the ends of the branches are coded to match STRUCTURE output.

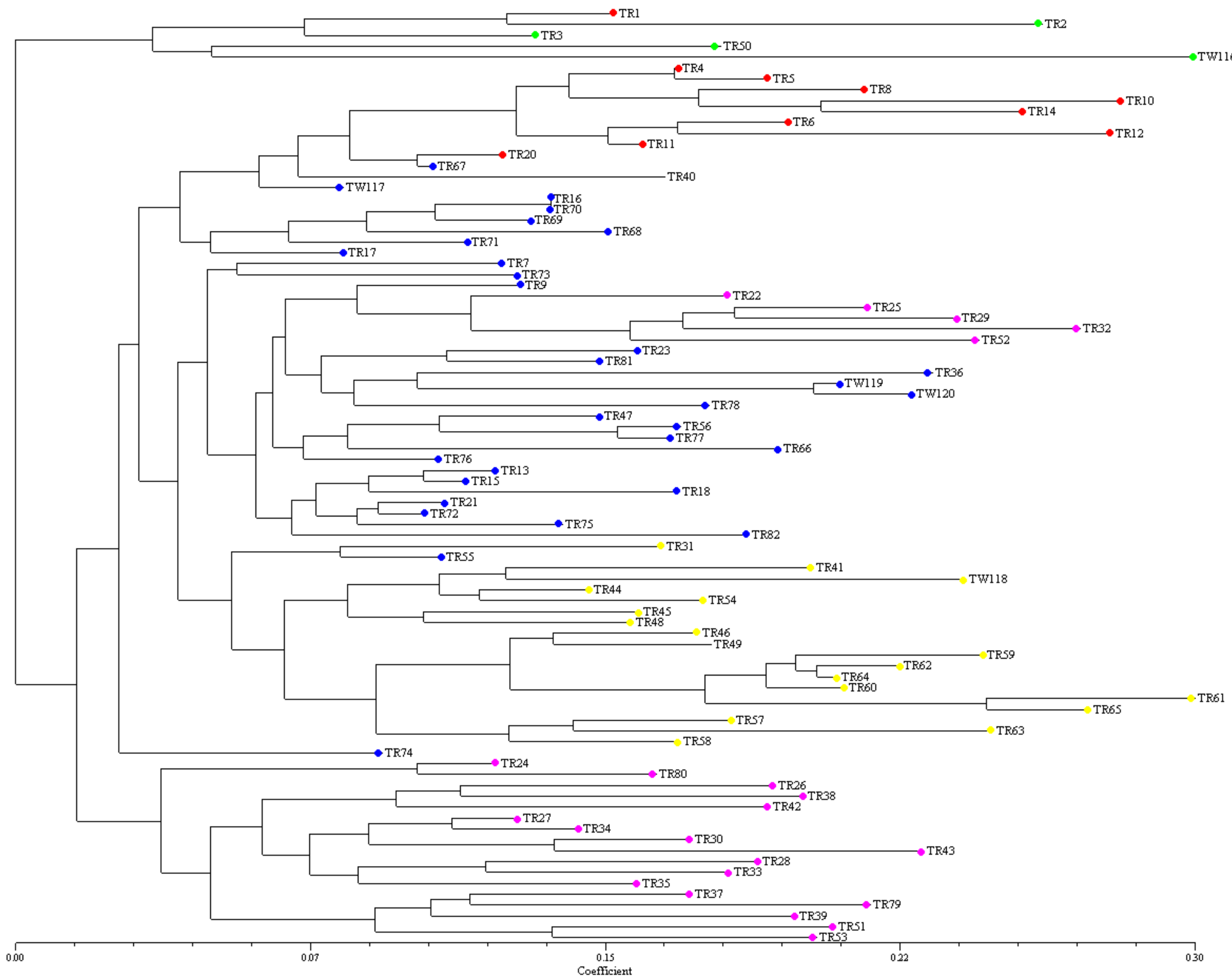


Figure 2.6 Neighbor-joining Phylogram. Dots located at the ends of the branches are coded to match STRUCTURE output.

Table 2.3 *N. rustica* Morphological Characteristics

Character	Overall Mean	Lowest Accession Mean	Highest Accession Mean
Plant Height	87.29 cm	TW118 (46.7 cm)	TR8 (155.9 cm)
Middle Leaf Length	22.96 cm	TW118 (10.9 cm)	TR5 (34.0 cm)
Middle Leaf Width	20.06 cm	TW118 (4.5 cm)	TR55 (30.0 cm)
Corolla Width	15.27 mm	TR67,TW118 (12.4 mm)	TR26,TR37 (19.7 mm)
Flower Length	23.55 mm	TR17 (20.9 mm)	TR37 (26.8 mm)
Capsule Diameter	10.68 mm	TR9 (0.78 cm)	TR63 (1.27 cm)
Days to Flower	44.27 days	* (28 days)	* (64 days)
Pre-topping PTA	1.38 %	TR27 (0.74 %)	TR29 (2.11 %)
Post-topping PTA	3.92 %	TW116 (0.74 %)	TR22 (6.94 %)

* Several accessions share these values. Refer to Appendix E.

Phenotypic data analysis

All of the morphological characters analyzed during the study were variable within accessions over years and replications and thus significant entry*year and entry*replication interaction terms were common (refer to Appendix A for ANOVA tables). For the characters plant height, middle leaf length and width, nearly all of the model terms were significant. These data suggest that these traits may have a sizeable genetic by environment interaction, thus requiring more replications or locations for accurate estimates of the true means of each accession. Although there was a significant entry by replication interaction factor in each analysis, capsule diameter, corolla width and flower length did not experience significant variation due to replication. It is possible that these traits may have less of an environmental component than the other quantitative variables that were recorded. Additionally, entry was the only significant main effect for days to flower. Variation between replicates was not expected for this trait since most *Nicotiana* species are known to exhibit photoperiod sensitive floral induction (Steinberg, 1959).

N. rustica alkaloid levels were significantly affected by topping the plants in 2006 ($F = 268.82$ with 1,85 df; $p < 0.0001$). This result was expected because alkaloids are known to be synthesized in the roots of *Nicotiana* species and to increase in concentration in the laminar tissue after the loss of apical dominance (Bush and Crowe, 1989). Furthermore, alkaloid biosynthesis and accumulation are also known to vary considerably with environmental conditions (Bush and Crowe, 1989). This is evident in this study by the fact that all of the main effect model terms for post-topping PTA are significant (entry, location, year, and replication). Additionally, pre-topping PTA, which was not significantly influenced by entry ($F = 0.80$ with 85,85 df; $p = 0.8472$), also had significant location and replication factors.

Morphological distances (DGOWER) ranged from 0.055 (between TR 13 and TR 14) to 0.711 (between TR 2 and TR 37), with a mean of 0.346. Principle component analysis of the morphological data captured 59% of the variation present in the data set within the first two components. When these two components were plotted against each other, a fairly uniform distribution of the accessions emerged, where only a few accessions appeared to be outlying (see Figure 2.7). When the accessions were identified by categorical variables, strong clustering patterns were seen for variety type (Figure 2.7 A), origin (Figure 2.7 B) and stem type (data not shown). For variety type, there is a clear distinction between the clusters of the brasilia and pumila types, with all of the accessions that were designated as intermediate in variety type lying between the two. The sole pavonii accession (TW 116) lies on the edge of the scatter plot but is not clearly separated from the other two variety types by this set of morphological data. Stem type, which is often used during variety classification, follows a pattern very similar to that of the variety identification. For accession origin, it can be seen that many of the accessions collected from the same region tend to cluster together, although it is not always the case (see accessions from Zambia). Only weak associations were seen between the quantitative morphological data and leaf shape or leaf surface, where there appeared to be a gradient from smooth to puckered but all of the classes that were not "smooth" were very mixed (data not shown).

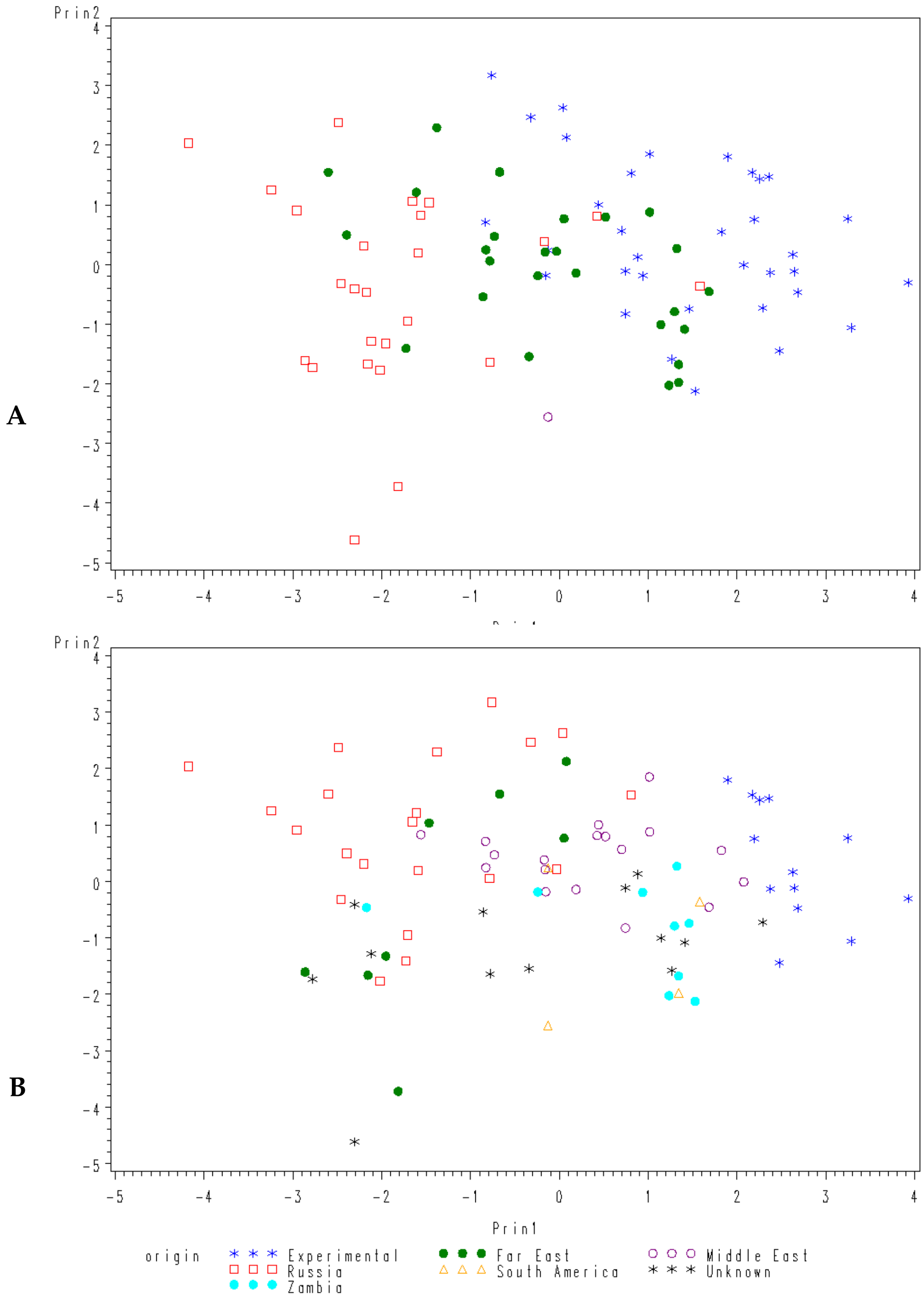


Figure 2.7 Principle Component Output 1. (A) When variety is mapped over the output, a clear clustering pattern can be seen among the accessions. (B) Mapping accession origin over the output reveals that there tends to be a correlation between accession origin and morphology.

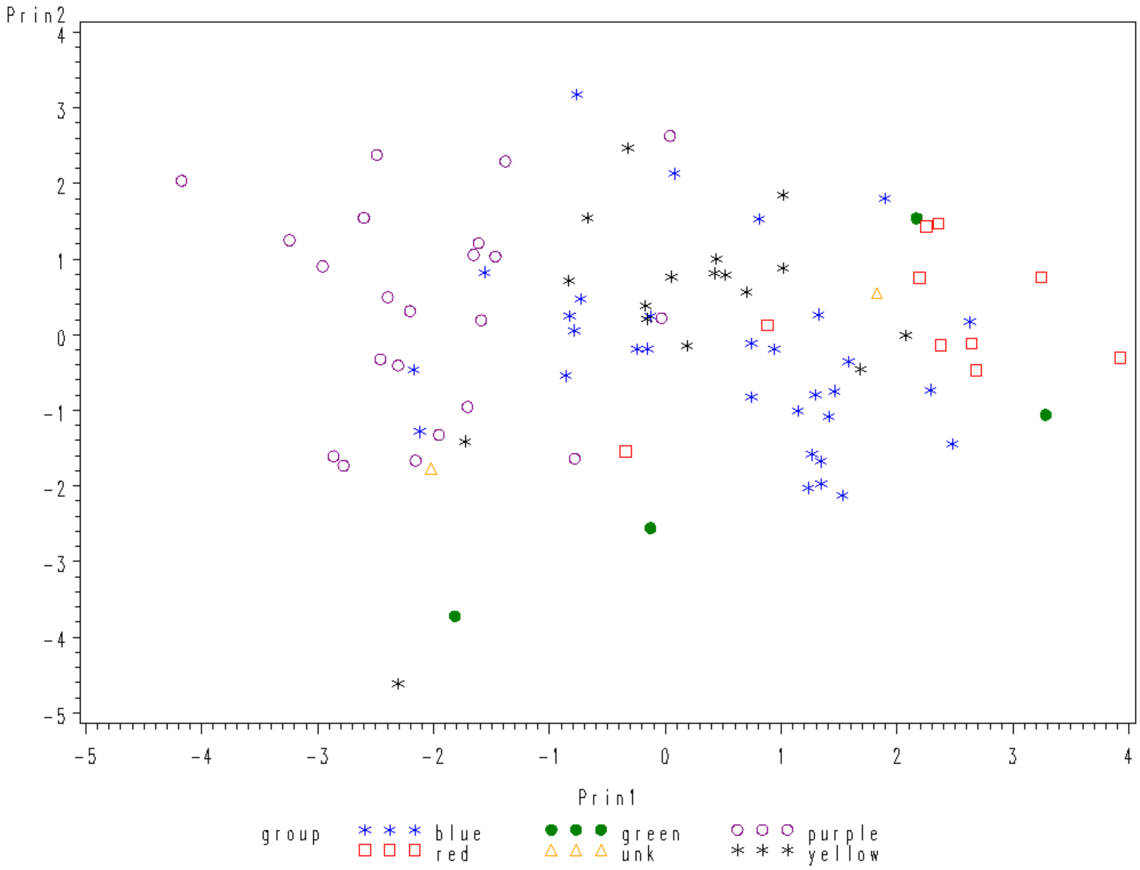


Figure 2.8 Principle Component Output 2. STRUCTURE groups were mapped over the morphological data principle component analysis in order to compare the genetic groups with the distribution of phenotypes. From the output it can be seen that there tends to be a correlation between the morphological characteristics of accessions within genetic populations, especially in the case of the purple, red and yellow populations.

DISCUSSION

Genetic Analysis

The lack of sequence variation in the *trnL-F* region was unexpected but it is not a singular phenomenon. A similar result was also reported by Jordan *et al.* (1996) when analyzing the chloroplast *rpL16* intron within species of duckweed. Although they had observed a high level of interspecific variation at the locus, they reported no variation in one duckweed species and only one sequence variant in another species. The lack of variation within *N. rustica* at the *trnL-F* region indicates that either the locus has a very low mutation rate, possibly due to some unknown function, or that *N. rustica* may have speciated recently and not yet had the time to accrue mutations in this region. Assuming the common neutral mutation rate of 10^{-9} per nucleotide site, the latter scenario may be more likely since Lim *et al*. (2007) revealed *N. rustica* to have speciated less than 200,000 years ago.

In order to proceed with phylogentic analyses based on genetic data obtained from AFLP loci, assumptions must first be made about the loci. These assumptions include independence between fragments (loci) and highly homologous DNA sequences between comigrating fragments of the same length, although the latter is not always satisfied. In diploid species of *Echinacea*, for example, the intraspecific sequence homology of bands at a monomorphic AFLP locus was shown to range from 79% to 99%, while there was only 31% to 45% homology at a polymorphic locus (Mechanda *et al.*, 2004). The data from the same study also suggest that the situation may be even more exaggerated in tetraploid species since *E. pallida*, a tetraploid, had even lower levels of intraspecific sequence homology at the same AFLP loci (76% and 24%, respectively). The incorrect assignment of homology between comigrating AFLP bands can lead to substantial amounts of homoplasy in the data set (Koopman, 2005). In addition to non-homology between fragments, homoplasy can also occur due to the independent loss of AFLP fragments among individuals.

Homoplasy due to non-homologous fragments can result in a form of long branch attraction during phylogenetic analyses (Koopman, 2005) and a general inability to reconstruct the correct topology representing the true history among individuals. It is believed that the significant amount of homoplasy present in the *N. rustica* AFLP data set may be due to a lack of sufficient sequence homology between the comigrating fragments and that this homoplasy resulted in the failure of the parsimony analysis. However, homoplasy is not the only aspect of AFLP data sets which acts to confound phylogentic analyses. Additional complications arise from the incorrect determination of (dis)similarity due to the dominant nature of AFLP markers, the presence of ancestral polymorphisms and the situation where high similarity between two individuals does not always represent a common ancestry (Meudt and Clarke, 2007). Additionally, there is asymmetry in the probability of changing from one allele state to the other such that it is more probable to lose a fragment than to gain a fragment (Koopman, 2005).

It is not surprising then, when considering these numerous complications, that the three different genetic data analyses that were performed failed to display convergence. A combination of the relatively low number of polymorphic loci that were available for analysis and the large number of individuals that were investigated could have additionally contributed to the lack of convergence. However, some similarities are present in the outputs of each of the analyses. The UPGMA-SAHN and Neighbor-joining clustering analyses, which are based on genetic distance measures, were able to reconstruct possible histories, despite the considerable homoplasy present in the original data set. As can be seen in Figures 2.5 and 2.6, there is a fair amount of agreement between the topology of a number of clades produced by the two phylogentic analyses. An example of this is the appearance of two monophyletic groups in both analyses, one containing TR 37, TR 39, TR 51, TR 53 and TR 79 and the other made up of TR 16, TR 68, TR 69, TR 70 and TR 71 (see *Inferences…* below for a more detailed discussion). However, larger clustering patterns do not tend to be consistently supported across the two analyses.

STRUCTURE did find significant population structure among the 86 *N. rustica* accessions based on their AFLP profiles. The five pools of germplasm produced by this analysis are generally in agreement with the larger clusters produced by the phylogenetic analyses. At first glance, the members of the populations appear to be completely random, especially when considering the phenotypic characteristics associated with individual accessions. A closer look at the AFLP profiles revealed five distinct populations, each with their own patterns of allele frequencies (see Figure 2.4). For example, the green population is "fixed" for the presence (1) of a band at locus 11, while this band is absent from all other populations. Likewise, at locus 20, the absence (0) of this band is approaching "fixation" in the purple population while it is still "segregating" in the yellow population. Common population terminology and concepts do not exactly fit this collection, however, since the accessions have been maintained within the collection via selfing—several for many generations. Thus each accession, most likely approaching homozygosity at all loci, has been subjected to its own genetic drift, independent of the other accessions.

Phenotypic Analysis

The mapping of variety type on the principle component analysis of the morphological data revealed a clear trend from the pumila variety, to intermediate forms, followed by the brasilia variety. This observation supports the idea of a continuum of variation within *N. rustica*, as was proposed by Goodspeed (1954). On one end of the continuum are the experimental lines generated by H. H. Smith, while the other end is comprised mainly of accessions from Russia, which were also known to be heavily bred and selected. It is interesting that TR 13, Brasilia No. 23, and TR 15, the *N. rustica* var. *brasilia* Schrank type strain, occupy the very middle of the chart. These accessions have been heavily cited in *N. rustica* research and Brasilia No. 23 is known to contribute to the pedigree of TR 18 (Smith and Bacon, 1941). It is possible that these accessions, with a handful of others that may or may not be in this particular collection, could be the sources of much of the variation that is seen in the collection today after three centuries of breeding and selection.

From the principle component analysis it is obvious that a few accessions have divergent morphologies, namely TR 37, TR 50 and TW 118 (and additionally the pavonii accession, TW 116, though it was not well differentiated by the variables in this study). With the exception of TW 118, TR 37 is the shortest accession, with flowers that are both longer and wider than almost all of the other accessions and it also flowers very early (less than 28 days after transplanting into the field). What distinguishes TR 50 from the rest of the accessions is not as obvious as a single characteristic. It shares small capsules with its nearest neighbors, TR 40, TR 41, TW 116 and TW 118, but does not have their elliptical leaves. It also shares very low alkaloid levels with TW 116 and TW 118, and additionally anthocyanin characteristics with TW 116. At one point, TR 50 had been designated *N. campanulata*, a distinct species of *Nicotiana*, until it was agreed upon by others to belong to *N. rustica* (Anderson, 1925; Reaber *et al.*, 1963). TW 118, designated in the collection as the variety pumila, was not used as a standard for the classification of the other accessions because, like TW 116, no other accession resembles it, with possibly the exception of TR 40. These results indicate that it may be advantageous to have a widespread re-evaluation of the species and develop a new varietal classification system.

Genetic and Phenotypic Correlation

The Procrustes analysis found a significant correlation between the molecular and quantitative measures of diversity, although it was very small (r = 0.2101). When looking at the raw data, however, pairs of individuals did not appear to have associated morphological and genetic distances. The lowest morphological distance is between TR 13 and TR 14 (0.055) and yet the same two accessions have a genetic distance of 0.310, which is larger than the average genetic distance between accessions (0.281). Additionally, when comparing TR 2 with TR 63, they have the largest genetic distance (0.547) but they have a morphological distance of only 0.236. It is possible that few genetic changes could be responsible for the gross morphological differences between *N. rustica* accessions. This seems unlikely, however, given that Ren and Timko (2001) were able to successfully differentiate agronomic classes of tobacco (analogous to variety as used in this study) with AFLP markers, although they used almost twice as many loci. On the other hand, changes in DNA methylation patterns were determined to be the main cause of genomic instability in synthetic allohexaploid wheat (Dong *et al.*, 2005).

Previous studies have had mixed reports about the significance and magnitude of the correlations between their morphological and genetic datasets. Tatineni *et al.* (1996) found a significant correlation (product-moment, r = 0.63) among the two configurations for

17 genotypes (including different species and interspecific hybrids) of cotton. Among landraces of plantains, Crouch *et al.* (2000) found no significant relationship between the two measures of diversity (Spearman's r = 0.12). In a large meta-analysis, Reed and Frankham (2001) found that the mean correlation of morphological and genetic measures of diversity over all of the population data sets included in their study was 0.217, consistent with the results of the current study. Factors which could result in a low correlation include non-additive genetic variation, differential selection, the impact of regulatory variation, different mutation rates, low statistical power and the effects of the environment on quantitative traits (Reed and Frankham, 2001).

Figure 2.8, the principle component analysis of the quantitative morphological data with the STRUCTURE groups mapped over it, also picks up on this small correlation between the genetic and morphological data. STRUCTURE analyzes a set of genetic data for a given group of individuals for population structure—subgroups of individuals which appear to belong to the same interbreeding population based on their allele frequencies. This differs from phylogenetic analyses which assume that all of the individuals are related by direct lines of descent. The analysis of the genetic data in STRUCTURE determined that there were five discrete populations within the sample. From the principle component output, it is clear that within these five populations, individuals do not only share similar patterns of alleles but they also tend to have similar morphological characteristics. However, this is a complex relationship where all of the individuals within a population are not united by a singular characteristic.

Inferences on N. rustica Accession Relationships

Accessions which have the probability of belonging to multiple populations were interpreted as an indication of a hybridization/recombination event of members of those populations. This is evident in TR 1 and TR 2 which are known to have been produced by controlled crossings, though their exact pedigrees are unknown. Each is as likely to belong to the green population as it is to the red population. Additionally, it is apparent from the analyses that either TW 116 or TR 50 was used in the breeding and selection experiments of H. H. Smith in the early 1940's, especially in the pedigrees of TR 1, TR 2, and TR 3. Based on some inferences made from available collection information, it is believed that TR 50 was not collected until circa 1957, and thus it is likely that TW 116 is indeed the green contributor to these accessions. It is also evident that at least two other diverse *N. rustica* parents were used to develop these experimental lines (TR 1 through TR 12). As TR 14 is known to have been in the collection as early as 1941, it is probable that this is the red contributor, but information about the origin of TR 20 is limited and its potential role cannot be excluded. The blue donor of the experimental lines cannot be determined from the analyses, though it is likely to be TR 15, TR 16, TR 17 or TR 18, as these are some of the oldest accessions in the collection and were heavily studied prior to 1950.

Analysis of the other accessions within the blue, purple and yellow STRUCTURE populations is not as intuitive. There is no readily visible correlation between any of the measured phenotypic traits and the members of a particular population. It is possible that more morphological, physiological or genetic investigations are needed to determine why these accessions are grouped together, or whether they truly belong together. All further discussion will be conducted assuming the correctness of the data at hand. The yellow group is comprised mainly of the *N. rustica* accessions collected from Pakistan and the purple population consists almost exclusively of the accessions from Russia, as well as those from New Zealand. The blue population is much more of a mixed bag but most notably contains all three of the oldest accessions (TR 15, TR 16 and TR 17). These accessions came to the US collection circa 1913 from Brazil by way of a turn of the century international collection in Italy. Interestingly, TR 70, now known to have an identical genotype with TR 16, was collected from a field in Zambia in 1981. In fact, all of the accessions collected from Zambia are also in the blue population and have profiles that are very similar. It is possible that one or all of the accessions from Italy were the founders of the Zambia population.

Five clusters of three or more accessions are supported by all three genetic analyses. Though members of the purple STRUCTURE group tend to clade together in both of the phylogenetic analyses, the specific topology of the relationships tends not to be the same except for in the case of TR 37, TR 39, TR 51, TR 53 and TR 79. The first two accessions are from Russia, the second two from New Zealand and the last one is a selection from TR 42 (believed to be from a botanical collection in France). These five accessions are all of the pumila variety, with smooth broad-elliptic to ovate leaves. Their leaves tend to be relatively small and they range from 3-4% alkaloids after topping, but these characters are not unique to these accessions. Visually, they appear very similar with the exception of TR 53 which lacks the dark green coloration of the other four accessions, appears more brushy and has smaller flower heads.

As noted previously, many of the accessions from Pakistan clade together, with the exception of TR 66 and those accessions collected in an earlier expedition (TR 45 through TR 48). This was even evident in the failed PAUP analysis, where it tended to be the only clade supported when consensus trees were generated. More specifically, two subgroups are supported by both the SAHN and neighbor-joining analyses. TR 57, TR 58 and TR 63 are morphologically very similar, though TR 63 has leaves which are noticeably more puckered. In addition to this, TR 57 and TR 63 both have high levels of resistance to race 1 of black shank. The other group that is recognized consists of TR 59 through TR 62, TR 64 and TR 65. TR 61 and TR 65 are shown as a pair separate from the rest of the group. Phenotypically this makes sense because, though they do not resemble each other, they are different from the rest of the group which tends to be brasilias and brasilia-like intermediates. TR 61 is an odd type of pumila with distinct acute leaf tips, while TR 65 is characterized by a very tall inflorescence and low alkaloid levels.

From the blue STRUCTURE group, there are two distinct clades that are supported by both phylogenetic analyses. TW 119 and TW 120, which are known to only differ at one AFLP locus, are related to TR 36. Interestingly, TR 36, which is from Russia, is clearly of the brasilia variety while TW 119 and TW 120 are of intermediate type. Visually, the accessions do not resemble one another. The branch lengths in both trees are long and this indicates that the accessions diverged early, which may account for the large differences in morphology. The members of another group, comprised of TR 16, TR 68, TR 69, TR 70 and TR 71, are much more closely related. The latter four accessions are from Zambia while TR 16 is originally from Brazil, although it was donated from a collection in Italy. This clade really points out the failure of the AFLP loci to adequately capture the genetic components of phenotype. Members of this clade are of the brasilia (TR 16), intermediate (TR 68, TR 69, TR 71) and pumila (TR 70) varieties. What makes this clade especially perplexing is the fact that TR 16 and TR 70 have identical AFLP profiles.

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CHAPTER THREE

Investigating *Nicotiana rustica* as a Potential Source of Disease Resistance for Cultivated Tobacco

ABSTRACT

Losses due to disease can have a major impact on the overall yield of a tobacco crop. Resistance to few diseases has been found within *Nicotiana tabacum* germplasm, and thus wild *Nicotiana* species have been screened extensively for sources of resistance. *N. rustica* has been evaluated as a potential source of disease resistance traits that could be introgressed into commercial tobacco (*N. tabacum*). Prior investigations of *N. rustica* identified resistance to black shank (caused by *Phytophthora nicotianae* var. *nicotianae*), *Tobacco Mosaic Virus* (TMV), and wildfire (caused by *Psuedomonas syringae* pv. *tabaci*). In these studies, the diversity of responses within the species was ignored, witnessed by only one or a few accessions of *N. rustica* historically being used during disease screens. In this study, 86 diverse accessions were examined to determine if there was any variation in pathogen responses that may have been neglected in previous studies. Although experimental inoculations confirmed that *N. rustica* is generally susceptible to many pathogens, responses were not always uniform across or within accessions. All accessions were found to have a hypersensitive response type of resistance to TMV. Additionally, all accessions were highly resistant or immune to black shank race 0. There was a high degree of variation in response to race 1, however, ranging from susceptible to highly resistant. Hybrids between *N. rustica* and *N. tabacum* were generated by using tetraploid K326 as the male parent and were subsequently backcrossed to K326. Resistance to TMV has been carried successfully into the second backcross.

INTRODUCTION

Field crops are continuously subject to losses due to pathogens and insect pests, even when the recommended management practices are followed. Tobacco (*Nicotiana tabacum* L.) is no exception to this. Losses due to disease can have a major impact on the profitability of a crop of tobacco by lowering the yield per acre and/or the quality of the leaf. Tobacco is host to a wide range pathogens including viruses, bacteria, fungi and nematodes, as well as a number of herbivorous insects. In 2005, infection due to target spot (*Rhizoctonia solani*) resulted in a 2.27% total value loss in flue-cured tobacco. Additional losses of 2.86%, 3.00% and 3.93% were due to black shank (caused by *Phytophthora nicotianae* Breda de Haan var. *nicotianae* G.M. Waterhouse), Granville wilt (caused by *Ralstonia solanacearum* (Smith) Smith) and *Tomato Spotted Wilt Virus* (TSWV) respectively, although annual losses due to TSWV have been as high as 6.3% (Sutton and Broadwell, 2006).

Adequate resistance to few pathogens has been found within tobacco germplasm, and thus wild *Nicotiana* species have been screened extensively for sources of resistance. In general, this has been a very successful practice. Cultivars are currently available with resistance to wildfire from *N. longiflora*, black shank resistance from *N. plumbaginifolia* and *N. longiflora*, resistance to *Tobacco Mosaic Virus* (TMV) from *N. glutinosa*, and black root rot resistance from *N. debneyi* (Clayton, 1969). However, not all sources of resistance from wild species can be readily transferred to *N. tabacum*. It becomes increasingly more difficult to transfer material from species which are distantly related to the diploid progenitors of *N. tabacum*. Additionally, the introgression of unadapted germplasm from wild species linked to the transferred resistance gene(s) has often led to adverse effects on yield and quality (Bai *et al.*, 1995; Chaplin and Mann, 1978).

Nicotiana rustica has been previously evaluated as a potential source of disease resistance to black shank, TMV, and wildfire (caused by *Psuedomonas syringae* pv. *tabaci* (Wolf and Foster) Young *et al.*) (Burk and Heggestad, 1966). In these previous studies, however, the diversity within this species was largely ignored as only one or a few accessions of *N. rustica* were used during disease screening. In this study, 86 diverse accessions were inoculated with TMV, TSWV, *Tobacco Etch Virus* (TEV), *Potato Virus* Y^{NN} (PVY^{NN}), *Ralstonia solanacearum* and *Phytophthora nicotianae* var. *nicotianae*. Since successful transfers of resistance genes from *N. rustica* to tobacco are known (Woodend and Mudzengerere, 1992), work was also initiated to transfer the *N. rustica* genes responsible for TMV and black shank resistance to *N. tabacum*.

MATERIALS AND METHODS

N. rustica Pathogen Inoculations

The material examined in these studies is consistent with the 86 *N. rustica* accessions presented previously in Table 2.1. Additionally, TR 19 of the *Rustica* collection (PI 499177), a *N. rustica-N. tabacum* hybrid of unknown origin, was included in these investigations as a crude reporter of trait transferability. *N. glutinosa* (PI 555507), *N. paniculata* and *N. undulata* (see Table 2.1) were included in TMV detached leaf tests. For Granville wilt and black shank field tests, the tobacco varieties Hicks and K346 were used as susceptible and resistant checks, respectively. NC 1071 was included as an additional check for race 0 resistance in the environmentally controlled, race specific black shank experiments. *P. nicotianae* var. *nicotianae* isolates were kindly supplied by Dr. David Shew, professor of Plant Pathology at NC State University. Viral isolates used include TEV 155a, PVY^{NN}, TSWV Hawaii L and the common strain of TMV.

TEV, PVY^{NN} and TSWV

For TEV, PVY^{NN} and TSWV inoculations, accessions were seeded in MetroMix (Sun Gro Horticulture Canada CM Ltd.) under germination domes in a growth chamber at 25 ± 2°C with a 12h light cycle. Four seedlings of each accession were transplanted individually to 2 5/8″ x 2 5/16″ cells in 3601 tray inserts (Hummert International). At approximately three weeks after transplanting, plants were inoculated by dusting two leaves of each plant with carborundum and then rubbing the viral suspension on the upper leaf surface with a paintbrush. Viral suspensions were prepared by grinding 1.0 g infected plant tissue with a mortar and pestle and resuspending it in 10 mL 0.1 M phosphate buffer (0.03 mol NaH_2PO_4, 0.7 mol Na_2HPO_4 in 200 ml H_2O) for TEV and PVY^{NN} or 10 mL of TSWV inoculation buffer (0.01 mol L^{-1} Tris-HCl at pH 7.8-8.0, 0.01 mol L^{-1} Na_2SO_3, 0.1% cysteine). Plants were kept for observation two to three weeks after inoculation.

Tobacco Mosaic Virus

The same procedure was carried out as above, with a slight modification. A small amount of carborundum was added to the viral suspension prepared for TMV rather than dusting it on the leaves to be inoculated. Viral suspensions, prepared from frozen TMV infected tissue, were suspended in 0.1 M phosphate buffer. Due to the frequent death of young inoculated plants, additional detached leaf tests were performed on mature plants of most accessions to confirm hypersensitive responses to infection.

Since *N. rustica* is known to form necrotic lesions upon infection with TMV that are similar to those of *N. glutinosa*, *N. rustica* was screened via PCR for the presence of *N*, a gene from *N. glutinosa* that confers a hypersensitive response form of resistance to TMV. Two primer pairs, published previously by Lewis, Milla and Levin (2005), were used: E1 + E2 (5′-ACCAGAATGATATGTTCCAC-3′ and 5′-GGACTCAACGTTAATTCTCTG-3′) and N1 + N2 (5′-CGTCGACACATTATGCCATC-3′ and 5′-GAGGGGTCTTACCCCATTGT-3′). Amplification was carried out in an Eppendorf Mastercycler in 20 μL volumes containing 50 ng of template DNA, 1 x ThermoPol Buffer (10 mM KCl, 10 mM $(NH_4)_2SO_4$, 20 mM Tris-HCl, 2 mM $MgSO_4$, 0.1% Triton ×-100; New England Biolabs), 10 μM of each primer (E1, E2 or N1, N2), 10 mM dNTPs and 1U of *Taq* DNA polymerase (New England Biolabs) with the following cycling program: 2 min at 95°C, followed by 35 cycles of 30 sec at 95°C, 30 sec at 55°C, 40 sec at 72°C, with a final extension of 5 min at 72°C. Products were run on 1% agarose gels and stained with ethidium bromide. DNA was isolated as described previously (refer to Chapter Two).

Granville Wilt

Plants were transplanted into a bacterial wilt nursery at the Tobacco Research Station in Oxford, NC during the 2007 season and cultivated according to the standard recommendations for flue-cured tobacco (Smith *et al.*, 2006; Smith and Wood, 2006). The experiment was planted in a randomized complete block design (RCBD) with three replications. The number of surviving plants was recorded three weeks after transplanting and again once every two weeks for 10 weeks (a total of 93 days after transplanting). From this data, an average days-survival index was calculated for each plot. This value is calculated by multiplying the number of live plants in a plot each reading (including the stand count) by the number of days it has been since the previous reading (or transplanting if it is the stand count reading). These values for the disease reading intervals are then added and the sum is divided by the total number of plants that were recorded during the stand count (usually twelve).

Black Shank

Field experiments were conducted in black shank nurseries at the Tobacco Research Station in Oxford, NC during the 2005 and 2007 growing seasons. Accessions were germinated in float trays and grown, transplanted and cultivated according to the standard recommendations for flue-cured tobacco (Smith *et al.*, 2006; Smith and Wood, 2006). Experiments were planted in RCBDs with four replications in 2005 and three replications in 2007. The number of surviving plants per plot was recorded three weeks after transplanting and continued once every two weeks for 10 weeks (a total of 93 days after transplanting). From this data an average days-survival index was calculated for each plot.

An additional experiment to determine the resistance of the *N. rustica* accessions to specific races of *P. nicotianae* var. *nicotianae* was performed at the NC State University Phytotron. The experiment was a split-plot design with four replications blocked by growth chamber. Each chamber served as a replicate. Pathogen race, either 0 or 1, was applied as the main plot factor and the 90 entries were randomized within each race. Seeds were germinated on peatlite under germination domes at 25°C with 16 hr light. Approximately two weeks after emergence, seedlings were transplanted to individual cells of 3601 inserts containing a 50:50 soil mixture of peat and sand. For each race, six seedlings per entry were transplanted and treated as a plot. After seven to ten days, the plants were inoculated with the appropriate pathogen race by pushing two oat grains infested with the pathogen into the soil and watering heavily. The day temperature was increased to 30°C while the night temperature remained at 25°C. Data collection began one week after inoculation and continued twice a week until 26 days post inoculation. An average days-survival index was calculated for each plot within pathogen race.

The inoculum was prepared by growing the pathogen in sterile Petri dishes containing carrot agar (50 mL organic carrot juice, 950 mL distilled water, 20 g agar) at room temperature in the dark. Sterilized oat grains were placed on the culture after three to four days of growth. Cultures were considered ready for use when hyphae could be seen covering most of the oat grains, generally 12 to 16 days.

Data Analysis

The average days-survival index data from the Granville wilt and black shank experiments (field data was pooled) was analyzed using SAS v. 9.1 (SAS Institute Inc.). Field experiments were analyzed in PROC MIXED where replication and year (black shank only) were random variables. Least square means for each entry were calculated and the *N. rustica* accessions were compared to the controls (Hicks, K346) by the Dunnett procedure. The split-plot growth chamber experiment for specific black shank race resistance was also analyzed in PROC MIXED where block (replication) and race*block were random variables. Since the interaction race*entry was significant, means comparisons were made in terms of this variable. Least square means for race*entry were calculated, sliced by race, and then compared to the checks using the Dunnett procedure.

N. rustica × *N. tabacum* Breeding Methods

Breeding methods followed those of Legg and Mann (1961). Autotetraploid *N. tabacum* was produced by generating plantlets from tissue culture of leaf midribs of the tobacco cultivar K326. Plants with leaves characteristic of tetraploids (designated tetK326) were selected and used during crossing. TetK326 was used as the male in crosses to numerous accessions of *N. rustica*, but germinable seeds were only obtained from crosses to TR 9, TR 30, TR 50, TR 51, TR 52, TR 53, TR 54, TR 61, TR 68, TR 73 and TR 80. TR 51 × tetK326 was lost to disease before reaching maturity. All interspecific hybrids were self sterile but retained low female fertility during backcrosses to diploid K326, with the exception of TR 9 × tetK326, which was sterile. Only hybrids derived from TR 50, TR 52, TR 53, TR 68, TR 73 and TR 80 were sufficiently fertile to produce sizeable BC_1F_1 populations. Plants with low levels of male fertility were present in these populations but were not conducive to seed production. The BC_1F_1 plants with the highest female fertility within each population were backcrossed again to K326, and additionally to Hicks, to generate BC_2F_1 populations.

***BC_2F_1* Disease Screens**

Twenty-four BC_2F_1 families, derived from TR 52, TR 68 and TR 80, were screened for TMV resistance. Twenty seeds of each line were seeded in MetroMix and placed under germination domes in a growth chamber at 25 ± 2°C with 12 hr light. Due to the low germination rate of some lines, three to eight seedlings of each line were transplanted into individual cells of 3601 inserts containing MetroMix. When the plants were approximately five weeks old they were inoculated with TMV by the method described previously.

A screen for resistance to race 1 of *P. nicotianae* var. *nicotianae* was also conducted on the BC_2F_1 population. Ninety-one BC_2F_1 families, K326, K346 and Hicks were entered into an unreplicated test at the NC State University Phytotron. Entries were germinated, transplanted and inoculated as described previously. However, again due to the low germination rates of some lines, four to twelve plants of each entry were transplanted. Observations were taken over 29 days in a similar manner to the race specific test. Additionally, a field experiment was planted alongside the *N. rustica* black shank experiment of 2007, but the data was excluded due to the small number of entries (9) and the extreme drought conditions that were present during the test (field not irrigated).

Figure 3.1 TEV Responses. (A) The reaction of TR 33 is shown as a representative of how most of the accessions reacted. (B) TR 61 depicts the necrotic inoculated leaves that was common among the accessions from Pakistan.

Results and Discussion

N. rustica Pathogen Responses

Tobacco Etch Virus

All 86 accessions of *N. rustica* tested showed no resistance to TEV infection, including TR 19, and were fairly uniform in the degree of symptoms caused by the pathogen. Common symptoms displayed included vein clearing, chlorotic mottling and leaf puckering leading to severe distortion (see Figure 3.1). However, a handful of accessions, primarily originating from Pakistan, displayed unique necrotic lesions on inoculated leaves. The lesions grew over time until eventually the whole leaf was necrotic. Nevertheless, these plants showed a severe systemic infection similar to all of the other accessions. It is possible that this could have been a type of hypersensitive response for which the necessary environmental conditions are not known, as was previously the case for the hypersensitive response to TMV infection. More investigations are needed to confirm this speculation however.

Even though the plants in the current study were not artificially wounded by puncturing, the results obtained were very similar to those previously described by Holmes (1946). He reported that the virus was recoverable, both locally and systemically, from *N. rustica*. Plants in his study also displayed no local symptoms but systemically displayed vein clearing, chlorotic mottling and distortion. It is evident that *N. rustica* is susceptible to TEV, regardless of the means of virus entry or the accession tested (except for the possibility of the aforementioned).

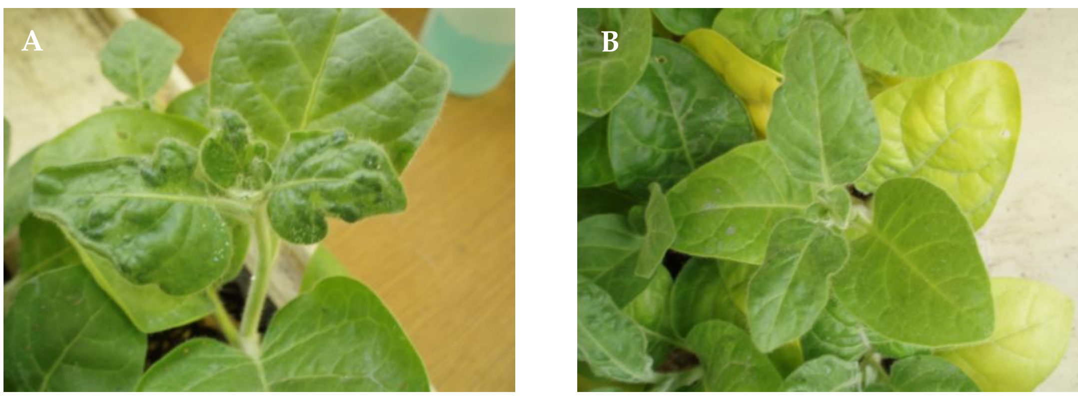

Figure 3.2 PVY^{NN} Responses. Differential responses of N. rustica accessions, ranging from rather severe leaf puckering (A) to almost asymptomatic (B).

Potato Virus Y^{NN}

Symptoms did not appear on TR 19 or any *N. rustica* accession infected with this potyvirus prior to two weeks post inoculation. When symptoms finally manifested, they included puckering that led to leaf distortion and a dark mottling (see Figure 3.2). The degree of these symptoms varied independently among accessions from mild to severe, where mottling was commonly mild. Variation was also seen among responses within accessions. All of the *N. rustica* accessions and TR 19 were resistant to the necrotic effects of

PVY^{NN}. The most tolerant accessions were TR 33, TR 35, TR 39, TR 40, TR 48, TR 51, TR 67, TR 70, and TW 118. However, it was observed that many of the accessions with milder symptoms were bolting and that slower growing plants tended to have more severe symptoms. An example would be TR 52, where the three plants bolting had very mild symptoms and the one that was not bolting had moderate symptoms. *N. rustica* exhibits day-length sensitive flowering and many accessions are induced to flower while in growth chambers. It is possible that the more rapid growth during bolting allowed the plants to temporarily outgrow the spread of the virus. Evidence for this comes from the fact that, in bolting plants, the first two leaves after those that were inoculated tended to have very mild symptoms (if any at all), but the third leaf was consistently more symptomatic.

A previous study by Sievert (1972) reported *N. rustica* to be susceptible to PVY due to the abnormal appearance of the infected plants, but there was no mention of which strain of PVY was used. Another study, which included thirteen different isolates of potyviruses (including strains of PVA, PVX and PVY), found both sensitivity and tolerance among the *N. rustica* accessions tested (TW 116 through TW 120) to three strains of PVY^{N} (van Dijk and Cuperus, 1989). These same accessions had varying combinations of mild and moderate symptoms during the current study, but comparisons cannot be made because no detailed description of symptoms was given by van Dijk and Cuperus, who only noted "symptomless systemic infection". Even though *N. rustica* is resistant to the necrotic effects of the PVY^{NN}, it appears that it would not be useful in a breeding program unless it was combined with another source of resistance that would diminish the mottling and puckering, which would reduce tobacco quality and yield if the *N. rustica* resistance was used alone.

Tomato Spotted Wilt Virus

All *N. rustica* accessions examined were highly susceptible to infection by TSWV, as was TR19. Symptoms included local and systemic necrotic ring spots and networks along with severe leaf deformation (see Figure 3.3). Occasional plants were also seen to be naturally infected with TSWV by thrips while growing in the field. Gardner and Whipple (1934) briefly reported *N. rustica* as a host for the spotted wilt of tomatoes, but no other reports were found. It is obvious however, that *N. rustica*, like many other plant species (e.g. *Solanum* spp., *Capsicum* spp. and *Arachis* spp.), has no resistance to this pathogen.

Figure 3.3 TSWV Responses. Figures A and B depict the severe infections that were experienced by all of the *N. rustica* accessions which were inoculated with TSWV.

Tobacco Mosaic Virus

Necrotic lesions, often accompanied by yellow halos, were formed locally by TR 19 and the 86 *N. rustica* accessions when inoculated with TMV (see Figure 3.4a). The response of the accessions was highly dependent upon the environmental variables, however, leading to numerous repetitions to confirm results. Necrotic lesions did not properly form if the temperature was too low and subsequent systemic infection was seen when the temperature was too high. However, temperature sensitivity is also known to be the case with *N* gene based resistance to TMV from *N. glutinosa* (Whitham *et al.*, 1994). The exact temperature range for proper lesion development was not determined during this study.

These local observations are in accordance with previous reports by Holmes (1946) and Diachun and Valleau (1954), however both studies also reported systemic necrotic spotting. It is worth noting that in both of the previous studies, the same description provided for *N. rustica* was also given for *N. glutinosa*, with Holmes considering both species to be susceptible. However, these studies were conducted before the hypersensitive response to TMV had been described. Neither study reported the temperature during experiments, which is known to be an important factor in TMV resistance (Whitham *et al.*, 1994), and Holmes also artificially wounded plants with 100 pin pricks during inoculations. It is possible that either the temperature was too high during the previous studies for these species to localize the virus.

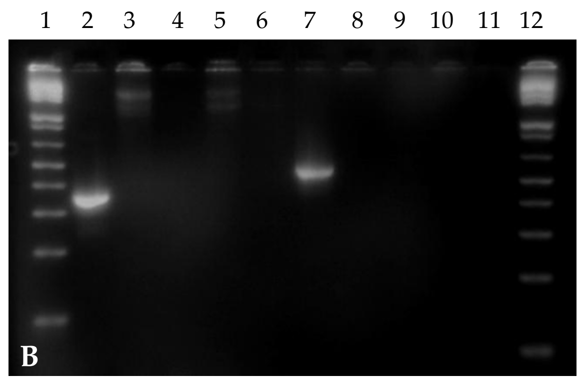

Figure 3.4 TMV Results. (A) Detached leaf results for *N. glutinosa*, and *N. rustica* accessions TW 118 and TR 56. (B) Results of PCR screen for the *N* gene. Lanes 1 and 12 are 1 kb ladder. Lanes 2-6 are amplifications with E1 + E2 while lanes 7-11 are from N1 + N2. Lanes 2 and 7 are *N. glutinosa*, 3 and 8 *N. undulata*, 4 and 9 *N. paniculata*, 5 and 10 *N. rustica* accession TW 116, 6 and 11 *N. rustica* accession TW 118.

N. glutinosa formed necrotic lesions during detached leaf tests but the accessions of *N. undulata* and *N. paniculata* tested did not. Since *N* gene mediated resistance is known to act as a single dominant gene, the proposed progenitor species of *N. rustica* were inoculated to try to elucidate which donor may have contributed resistance to TMV infection. It was hypothesized that the resistance came from the U genome (*N. undulata* like) donor because it has been recently shown that *N. undulata* shares a maternal common ancestor with *N. glutinosa* (Clarkson *et al.*, 2004). Although the data from the current study do not support this, it was discovered that both necrotic (resistant) and mottling (susceptible) forms of *N. undulata* exist (Holmes, 1936). Though a susceptible seed stock was used in this study, the fact that necrotic strains of *N. undulata* exist still points to a U genome donor of TMV resistance.

However, there is one report of a necrotic reaction in *N. paniculata* (Diachun and Valleau, 1954). When Holmes (1936) transferred the *N. rustica* gene responsible for the necrotic response to TMV to *N. paniculata*, he noted that since the resulting plants were "self-fertile, and would be identified as *N. paniculata* if found growing in the wild, … the new strain may for practical purposes be considered a necrotic-type variety of the species *N. paniculata*." It is therefore possible that seed stocks of this unnatural "*N. paniculata*" may have been passed around as true *N. paniculata*. Consequently, since most studies found *N. paniculata* to be susceptible to TMV (Holmes, 1936 and 1946; Kostoff, 1937), this one account of necrotic *N. paniculata* is not considered to be sufficient evidence to detract from a U genome origin of *N. rustica* TMV resistance.

Holmes (1936) referred to the gene he transferred from *N. rustica* to *N. paniculata* that was responsible for the localization of TMV in necrotic lesions as *N*. The label was then adopted for the gene responsible for the hypersensitive response to TMV from *N. glutinosa*. The PCR screen to investigate if *N* was the same in both cases resulted in successful amplification in *N. glutinosa* with both primer pairs, but not in *N. paniculata*, *N. undulata* or the accessions of *N. rustica* investigated (Figure 3.4b). Even though the accession of *N. undulata* was known to be susceptible, the failure of amplification in *N. rustica* confirmed that the gene is not identical in the two species. Although *N. undulata* and *N. glutinosa* share a common ancestor, they have undergone significant divergence since speciation (see branch lengths in Clarkson *et al.*, 2004). It is possible that the TMV resistance locus could have evolved independently in the two species such that the allele present in ancestral *N.*

undulata at the time of the hybridization producing *N. rustica* may have no longer resembled that of *N. glutinosa*. More analyses will be needed to determine whether the functional domains of the gene products have remained the same.

Figure 3.5 Granville Wilt Field Test. Nine plots of *N. rustica* accessions were planted in the area of the field that is bare, representing the total plot loss that was common. The tobacco plants that are in the background are the susceptible check, Hicks.

Granville Wilt

All *N. rustica* accessions tested performed significantly worse than K346 (average days-survival index of 87.65 days), with an average difference of 47 days. The *N. rustica* accessions also performed significantly worse than Hicks (average days-survival index of 64.14 days), by an average of almost 26 days, with the exception of TR 12, TR 18, TR 19, TR 20, TR 67, TR 74, TR 75, TR 76, TR 80, TR 82 and TW 117 which were not significantly different from Hicks. Most of the *N. rustica* accessions had experienced greater than 50% loss by seven weeks after transplanting compared to an average loss of 30% for Hicks at that time. Additionally, the pathogen load in the field was determined to be low due to the observation that the Hicks included in the experiment did not experience as much infection as expected (see Figure 3.5). The current study confirms the results of Clayton and Smith (1942) who reported *N. rustica* as highly susceptible. For a detailed listing of average days-survival indices refer to Appendix D.

Black Shank

During the field studies, all of the 86 *N. rustica* accessions and TR 19 survived significantly better than Hicks (average days-survival index of 52.84 days), at a 95% confidence level, by an average of 36 days. Additionally, twenty-five accessions, and the hybrid TR 19, survived significantly better than K346 (see Table 3.1), while all of the remaining accessions were not significantly different from K346. In fact, only four accessions (TR 2, TR 27, TR 50 and TW 116) had average days-survival indices less than that of K346 (differences of -2.68 days, -5.32 days, -6.62 days and -1.90 days, respectively). It is interesting that the *N. rustica-N. tabacum* hybrid, TR 19, had the highest overall average days-survival index (93.00 days) during the field experiments, surviving the whole duration of both seasons without any loss due to disease.

In contrast to the results of Litton *et al.* (1970), all but three accessions of *N. rustica* (TR 51, 21.33 days; TR 77, 21.71 days; TW 116, 18.75 days) had high levels of resistance to race 0 of the pathogen based on average days-survival indices from growth chamber experiments (see Appendix D for data). In fact, the hybrid TR 19 and 24 different *N. rustica* accessions experienced no loss during the course of the experiment. In this sense, their level of resistance was equivalent to NC 1071, which has qualitative resistance to race 0 of *P. nicotianae* var. *nicotianae* conferred by the *Ph* gene from *N. plumbaginifolia* (Johnson *et al.*, 2002). However, unlike the qualitative resistance of NC 1071, which is easily overcome by race 1 of the pathogen, high levels of resistance to race 1 are also present among several accessions of *N. rustica* (see Table 3.2).

Table 3.1 Black Shank Field Survival. *N. rustica* accessions with significantly higher average days-survival indices than K346.

Entry	Difference with K346 (days)	Dunnet-Hsu Adjusted P-value
TR19 (hybrid)	9.36	0.002
TR6	8.35	0.013
TR7	7.65	0.039
TR12	8.85	0.005
TR14	7.93	0.025
TR16	8.54	0.009
TR20	9.02	0.004
TR21	8.52	0.009
TR31	7.85	0.029
TR35	8.11	0.019
TR43	8.46	0.010
TR44	7.54	0.046
TR45	7.84	0.029
TR47	7.58	0.043
TR48	9.18	0.003
TR49	8.14	0.018
TR52	7.79	0.031
TR54	8.67	0.007
TR55	7.56	0.044
TR57	8.25	0.015
TR69	8.58	0.009
TR70	7.67	0.038
TR71	8.62	0.008
TR74	7.77	0.032
TR76	8.64	0.008
TW117	7.63	0.040

Figure 3.6 Black Shank Field Test. *N. rustica* accessions can clearly be seen surviving significantly better than the susceptible check Hicks (foreground).

There was a broader range of resistance among the *N. rustica* accessions to race 1 of the pathogen during growth chamber experiments, with average days-survival indices ranging from 13.33 to 25.42 days. While nineteen accessions were found to not be significantly different than Hicks, no accessions were found to be significantly different from K346 (see Appendix D for details). Additionally, no immune accessions were observed, as was the case with race 0. However, as can be seen in Table 3.2, eleven *N. rustica* accessions survived on average three to five days longer than K346 in the presence of race 1, though

Table 3.2 Race Specific Black Shank Survival. *N. rustica* accessions with immunity to race 0 and high levels of resistance to race 1.

	Average Days-survival Indices (days)	
Entry	**Race 0**	**Race 1**
Hicks	9.75	10.71
NC 1071	26.00	8.29
K436	25.71	19.77
TR19 (Hybrid)	26.00	20.11
TR6	26.00	23.58
TR12	26.00	23.94
TR16	26.00	24.75
TR18	26.00	23.13
TR20	26.00	25.42
TR21	26.00	24.58
TR22	26.00	24.02
TR28	26.00	23.42
TR37	26.00	23.33
TR54	26.00	23.97
TR80	26.00	23.00

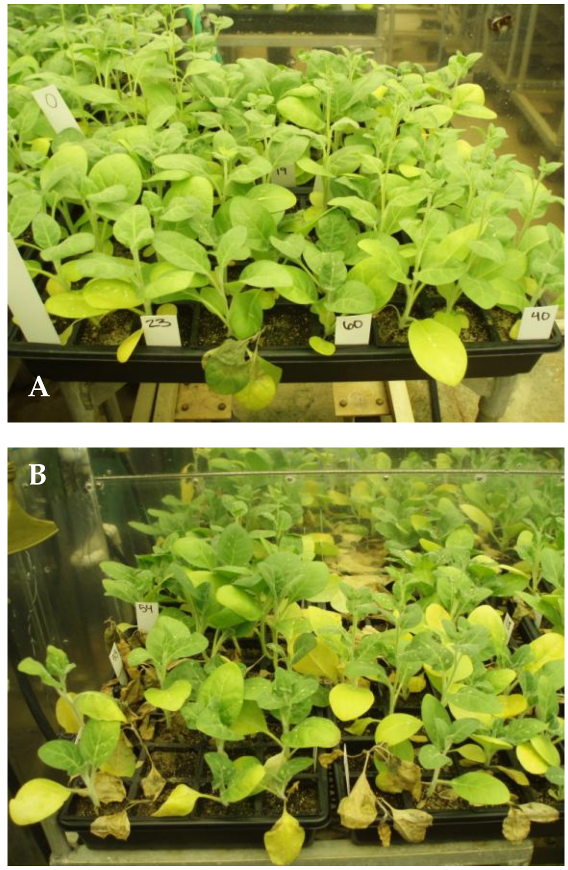

Figure 3.7 (Right) Race Specific Black Shank Experiments. While levels of resistance to race 0 were uniformly high among the *N. rustica* accessions (A), the response to race 1 was more varied and infection was common.

these differences were not found to be significant in this study. Further testing with greater replication will be needed to confirm the relative strengths of the quantitative resistance of K346 and these better performing *N. rustica* accessions.

These results are similar to the race 1 results of Litton *et al.* (1970), even though there was not agreement among the race 0 results. However, the results of the current study stand in heavy contrast to those of Li *et al.* (2006). They reported that the nine accessions of *N. rustica* they screened had only 0-32% healthy plants when inoculated with race 1. In the current study, a range of 4-92% healthy plants was observed, with more than half of the accessions with 50% or more (data not shown). A comparison of the three accessions that the studies had in common revealed that when plants are transplanted into individual cells (as in the current study), where competition is absent and healthy root systems are allowed to form, they resist the pathogen better (see Table 3.3).

The results of the current study confirm that certain accessions of *N. rustica* exhibit a wide range of partial resistance to *P. nicotianae* var. *nicotianae* and could in fact be a source of black shank resistance genes for commercial tobacco. Regardless if the highest level of *N. rustica* resistance is determined to be equivalent to that of K346, the deployment of varieties with *N. rustica* derived resistance could be a useful addition to the current sources of black shank resistance (i.e. *N. longiflora*, *N. plumbaginifolia*, and Fla 301 based resistance). Recent studies have shown that an increase in the genetic diversity of resistance mechanisms may help to ensure the long term effectiveness of each form of resistance by presenting the pathogen with more than one form of pressure to overcome (Mundt, 2002). This phenomenon has been documented in barley (Chin and Wolfe, 1984) and wheat (Mundt *et al.*, 1999).

Table 3.3 Comparison of Race 1 Results with Li *et al*. (2006).

		Percent Healthy Plants	
Entry	**PI Number**	**Li *et al.***	**Present Study**
TR1	499162	0	36
TR2	499163	18	25
TR13	499174	24	52

Observations on Interspecific Hybrids and Their Derivatives

Hybrids

While many crosses resulted in only a single fertile seed, three crosses (TR 50, TR 53 or TR 61 × tetK326) were sufficiently fertile that a small plot was planted in the field for observations. As noted by Shizukuda and Nakajima (1982), the variety of *N. rustica* used in the cross did not produce any gross morphological differences among the hybrids. Plants ranged from 168 to 225 cm in height with a mean of 194 cm, including the inflorescence.

Their stalks were thick and woody and the foliage was the dark green coloration that is common of *N. rustica*. Leaves were an average of 53 cm long by 35 cm wide and were similar in shape to those of *N. tabacum*. Apical dominance was generally maintained until topping. The alkaloid levels ranged from 1.42-2.42% pre-topping to 2.22-4.08% two weeks after topping.

Figure 3.8 *N. rustica* × TetK326 Hybrids. (A) TR 50 × tetK326 growing in the field. (B) A comparison of flower morphology among the interspecific hybrids and their parents.

The other hybrids were grown in the greenhouse for pollinations. As was the case in the field, they were largely uniform in appearance. They also grew vigorously (often reaching over 2 m in height) and flowered profusely. However, plants were not as hardy as those grown in the field and the leaves, though similar in shape, did not become nearly as large. Flower morphology was intermediate between the two parents (see Figure 3.7b), a feature that has been commonly reported by others (Shizukuda and Nakajima, 1982; Hamill *et al.*, 1985). Only a few differences were easily recognizable between hybrids. The hybrid derived from TR 61 had nearly white flowers which were only seen to be slightly pink when they first opened. The most distinct difference was the anthocyanin in the ovaries of the hybrid from TR 50, which was also seen in the coat of the capsule and the early stages of corolla development and occasionally in the floral stems. It is evident that it must be a dominant characteristic since it is readily expressed in the haploid condition. It has been postulated to be controlled by either one or two genes (summary present in Kostoff, 1943).

In general, however, the morphology of *N. rustica-N. tabacum* hybrids appears to be very consistent, no matter the varieties used in the cross or the method used to generate the hybrids. The hybrids produced by Shizukuda and Nakajima (1982) and Hamill *et al.* (1985), as well as those of Douglas *et al.* (1983), were all true hybrids with 48 chromosomes and equal portions of *N. rustica* and *N. tabacum* germplasm. The hybrids from the current study however, were sesquidiploids with two times more tobacco germplasm than *N. rustica* germplasm and yet, by all accounts were very similar to those of the aforementioned studies. This might indicate that a diploid copy of the tobacco genome may have no more impact on the resulting phenotype than a haploid copy.

BC_1 Population

The BC_1 population was very polymorphic due to the random assortment and loss of chromosomes, assumed to be predominantly from *N. rustica*. Pandeya *et al.* (1986) noted that their BC_1 population displayed morphological characteristics of both parents. This was also true of the population from the current study. While many plants were still dark green, some plants of a lighter green were seen. There were many leaf abnormalities, most notably strange patterns in vein formation, though no leaf tumors were seen. Many plants had leaves which were petioled with thin strips of laminar tissue connecting to the stem, but sessile leaves were also seen. Large variations in plant height were seen, but this may have been confounded by photoperiod sensitivity for floral induction. One rosette plant was seen, which though it had nearly a dozen leaves was no more than 30 cm tall. Figure 3.8 depicts some of the floral variation that was seen, including variations in length and color. A wide range of pistil lengths were also observed which appeared to share no correlation with corolla or stamen length, as highly recessed pistils were seen as well as one plant in which the pistils surpassed the stamens as well as the limb of the corollas. Evaluations of pollen viability by staining with acetocarmine revealed that though many plants had 0-10% viability, a few plants were seen to have as much as 50% pollen viability. However, this pollen failed to set any significant number of seeds when used in pollinations.

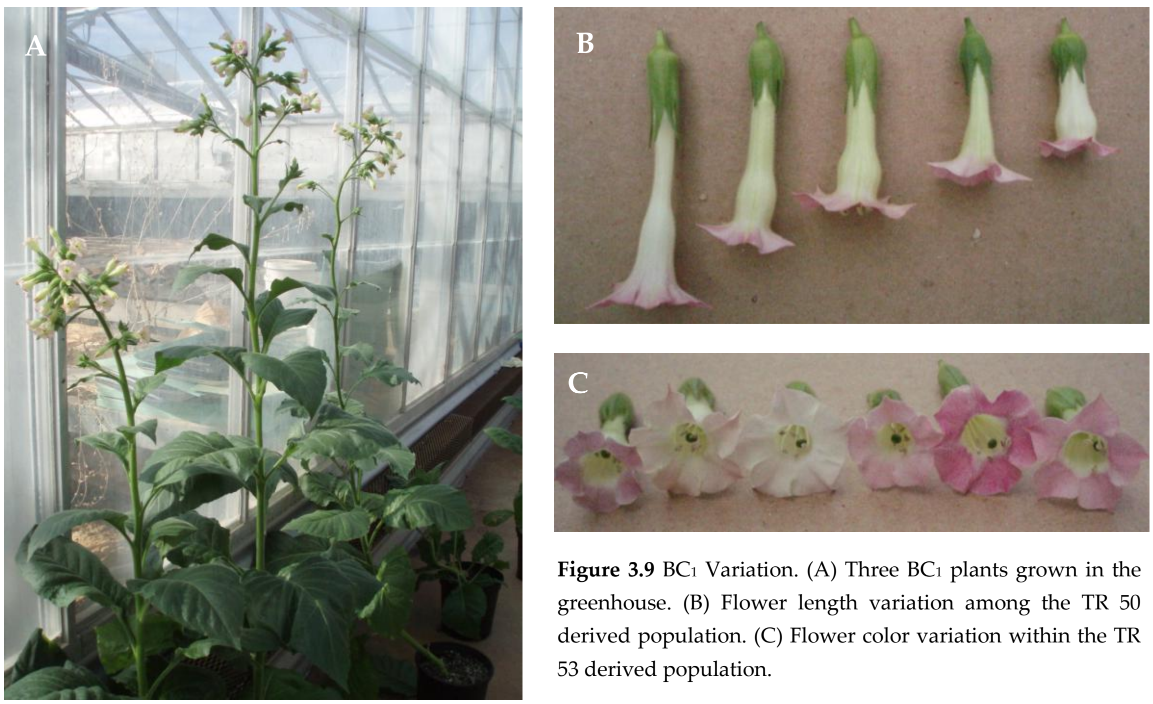

Figure 3.9 BC_1 Variation. (A) Three BC_1 plants grown in the greenhouse. (B) Flower length variation among the TR 50 derived population. (C) Flower color variation within the TR 53 derived population.

BC_2 Population

A substantial number of plants from this population were observed while growing in the black shank nursery at Oxford, NC. Besides occasional plants which had adverse developmental abnormalities, off type plants were less common than in the BC_1 population, where almost each plant was unique. Most of the plants had a compact form, though that could have been a result of the drought conditions. One obvious plant was seen in the field

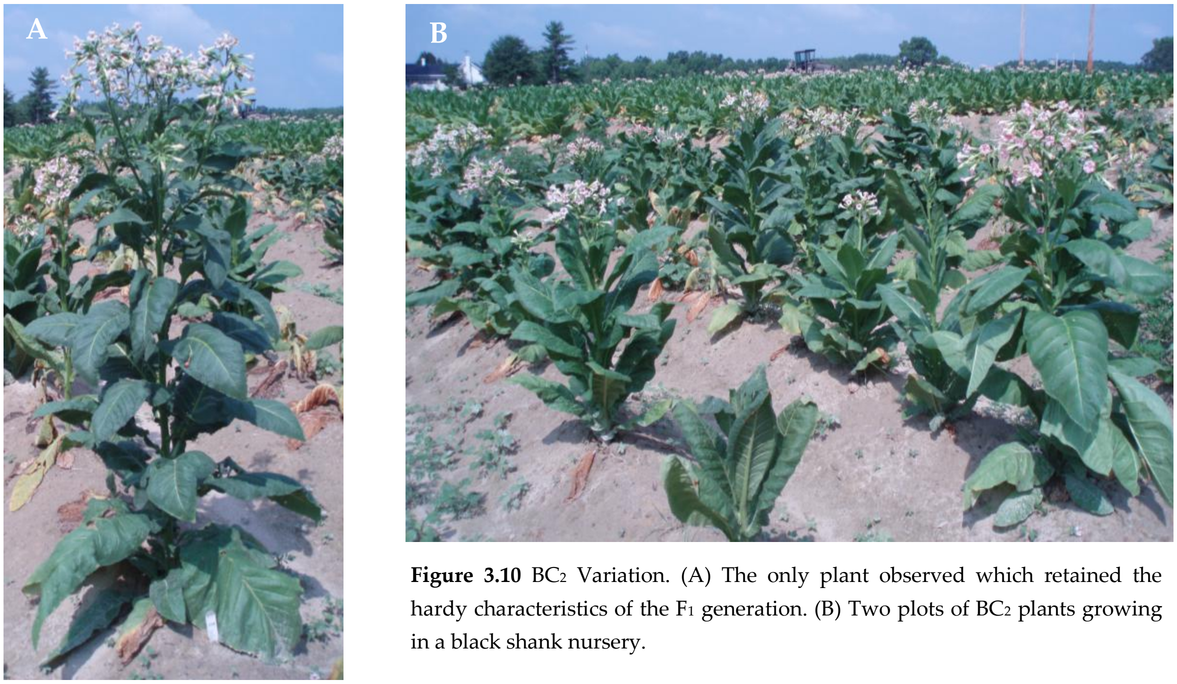

Figure 3.10 BC_2 Variation. (A) The only plant observed which retained the hardy characteristics of the F_1 generation. (B) Two plots of BC_2 plants growing in a black shank nursery.

which more closely resembled the hardy hybrids, with dark green foliage and woody stem. It did not have a compact shape and seemed not to be suffering from the water stress conditions. Floral morphology appeared to be much more consistent, with flowers tending to resemble more closely those of *N. tabacum*.

Transfer of Disease Resistance Traits

Tobacco Mosaic Virus

Differential responses were seen when BC_2F_1 plants were inoculated with TMV. Variability was also reported by Kostoff (1937) in the responses among his *N. rustica* × *N. tabacum* hybrids (F_1), which was dependent on which varieties were used in the cross. In general, five different classes of local responses were observed; brown necrotic lesions, brown necrotic lesions accompanied by yellowing, grayish necrotic lesions that resembled tissue bruising but were circular and expanding, yellowing with no necrosis, or no local response (see Figure 3.10). The latter two categories were not considered resistant during scoring. As expected, no lines were fixed for resistance, though a number of lines had apparently already lost the chromosome responsible for the hypersensitive response to TMV because all of the plants screened failed to form necrotic lesions of any type. An interesting phenomenon was additionally observed among the plants which were thought to be susceptible. BC_2F_1 plants which had not displayed any local response also failed to develop any systemic TMV symptoms by four weeks after inoculation, reminiscent of the heavily studied Ambalema type of resistance to TMV.

Figure 3.11 Responses of BC_2 Population to TMV. (A) Starting in the upper right hand corner and going clockwise, necrotic lesions, necrotic lesions accompanied by yellow halos, and grayish necrotic lesions. (B) Two BC_2 plants displaying yellowing on the inoculated leaves without necrosis.

A possible explanation for these varying responses may come from Holmes (1936). He selfed plants after the fourth backcross (BC_4F_2) of the hybrid *N. paniculata* × *N. rustica* to the recurrent male parent, *N. paniculata*, and two classes of necrotic lesion forming plants emerged—those that had "unmodified necrosis" and those that had "a delayed necrotic-type response associated with peripheral yellowing of lesions." Holmes calculated exactly a 3:1 ratio of unmodified to delayed necrosis and concluded that "delay of necrosis, with attendant yellowing of surrounding tissue, is dependent on a recessive gene (*d*, delayed necrosis)" and that it segregated independently of the chromosome bearing *N*. Additionally, since the delayed necrosis phenotype was not seen in the five previous generations, he determined that the trait must have been derived from *N. rustica*. Holmes however could not account for the deviation from a 3:1 ratio of necrotic forming progeny (including both unmodified and delayed) in this generation.

The observations of the current study, however, indicate a much more complicated system than could be seen with two interacting genes alone. The two gene system may account for the presence or absence of necrotic lesions with and without yellowing, but does not explain the other categories of response, including the Ambalema-like response. *N. rustica* chromosomes, and *N. tabacum* chromosomes with less probability, are being shed each generation which results in novel combinations of genes which could be responsible for the variability of responses to TMV. This system may provide a unique way to further study the genes and the pathways leading to resistance to this virus. It will be interesting to see if the Ambalema-like resistance will be stably transferred or if it will break down. Although the previously studied Ambalema resistance was commonly used in tobacco breeding, the tobacco lines generated always suffered from undesirable manufacturing qualities (Valleau, 1952).

Black Shank

Since a replicated test was not performed, the results will be briefly discussed in terms of the raw data without regard to significances. Nine BC_2F_1 lines, as well as K346, experienced no death due to infection of the pathogen during the experiment (see Appendix D for data). Thirty other lines had very high levels of survival, with a per plant average day survivals of 26 days of greater (90% of the total experiment). However, overall levels of infection were much lower in this test than in previous tests, and K346remained free of disease. More replicated tests of the lines are needed to better determine their true levels of resistance. However, from the current data it can easily be seen that the family derived from TR 80 has the best overall resistance. While individual lines from each family may have had high levels of survival, the families did not fair as well when considered as a whole. It was hypothesized when beginning this experiment that TR 80 derived lines would perform the best since of all the *N. rustica* accessions from which hybrids were derived, TR 80 had the highest level of resistance to *P. nicotianae* var. *nicotianae* (see Table 3.4). Along the same lines it was believed that the TR 50 family would be the worst performer, but that is not immediately evident from the data obtained.

Table 3.4 Comparison of Black Shank Race 1 Survival between *N. rustica* Accessions and Their Derived BC_2 Families. Race specific test is out of 26 days while BC_2 test is out of 29 days.

	Race 1 Average Days-survival Indices (days)	
Entry	**Race Specific Test**	**Derived BC_2 Family Mean**
TR50	13.33	23.52
TR52	22.44	25.91
TR53	18.78	24.34
TR68	20.34	21.80
TR73	20.63	24.60
TR80	23.00	26.56

Based on our data, it appears that resistance to black shank can be successfully transferred to commercial tobacco from *N. rustica*. While the material derived from TR 80 seems to be a good candidate for further studies, it may not represent the spectrum that is possible. As can be seen in Table 3.2, there are four other accessions (TR 16, TR 20, TR 21, TR 22) with extremely high levels of resistance to race 1 and with apparent immunity to race 0. In parallel with continued work towards the introgression of TR 80 germplasm, efforts should also be made to generate interspecific hybrids from these other accessions, especially TR 20, for the purpose of transferring their superior resistance.

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APPENDICES

APPENDIX A

ANOVA Tables for Morphological Data

ANOVA Dependent Variable: Plant height

Number of Observations Read	2579
Number of Observations Used	2551

Class	Levels
entry	86
year	2
rep	2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	343	1638506.720	4776.988	39.70	<.0001
Error	2207	265548.783	120.321		
Corrected Total	2550	1904055.503			

R-Square	Coeff Var	Root MSE	height Mean
0.860535	12.64851	10.96910	86.72246

Source	DF	Type I SS	Mean Square	F Value	Pr > F
entry	85	1470986.431	17305.723	18.49	<.0001
year	1	19480.810	19480.810	20.82	<.0001
rep(year)	2	4830.573	2415.287	6.45	0.0020
entry*year	85	79549.747	935.879	2.50	<.0001
entry*rep(year)	170	63659.158	374.466	3.11	<.0001

ANOVA Dependent Variable: Middle leaf length

Number of Observations Read	2579
Number of Observations Used	2500

Class	Levels
entry	86
year	2
rep	2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	343	82615.2483	240.8608	22.86	<.0001
Error	2156	22713.1541	10.5349		
Corrected Total	2499	105328.4024			

R-Square	Coeff Var	Root MSE	llength Mean
0.784359	14.13356	3.245745	22.96480

Source	DF	Type I SS	Mean Square	F Value	Pr > F
entry	85	70887.23550	833.96748	11.03	<.0001
year	1	188.13724	188.13724	2.49	0.1183
rep(year)	2	213.88329	106.94165	3.71	0.0265
entry*year	85	6429.12634	75.63678	2.63	<.0001
entry*rep(year)	170	4896.86596	28.80509	2.73	<.0001

ANOVA Dependent Variable: Middle leaf width

Number of Observations Read	2579
Number of Observations Used	2499

Class	Levels
entry	86
year	2
rep	2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	343	94486.5292	275.4709	11.86	<.0001
Error	2155	50053.1570	23.2265		
Corrected Total	2498	144539.6863			

R-Square	Coeff Var	Root MSE	lwidth Mean
0.653706	24.02461	4.819390	20.06022

Source	DF	Type I SS	Mean Square	F Value	Pr > F
entry	85	76156.99355	895.96463	7.76	<.0001
year	1	358.31718	358.31718	3.11	0.0814
rep(year)	2	637.91361	318.95680	7.21	0.0010
entry*year	85	9808.88428	115.39864	2.61	<.0001
entry*rep(year)	170	7524.42062	44.26130	1.91	<.0001

ANOVA Dependent Variable: Corolla width (2005 data only)

Number of Observations Read 1293

Number of Observations Used 1208

Class	Levels
entry	86
rep	2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	171	5039.387898	29.470105	11.38	<.0001
Error	1036	2682.463095	2.589250		
Corrected Total	1207	7721.850993			

R-Square	Coeff Var	Root MSE	cwidth Mean
0.652614	10.53556	1.609115	15.27318

Source	DF	Type I SS	Mean Square	F Value	Pr > F
entry	85	4305.467410	50.652558	6.07	<.0001
rep	1	24.911268	24.911268	2.99	0.0874
entry*rep	85	709.009220	8.341285	3.22	<.0001

ANOVA Dependent Variable: Flower length (2005 data only)

Number of Observations Read 1293

Number of Observations Used 1207

Class	Levels
entry	86
rep	2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	171	2867.617595	16.769694	7.27	<.0001
Error	1035	2386.998810	2.306279		
Corrected Total	1206	5254.616404			

R-Square	Coeff Var	Root MSE	flength Mean
0.545733	6.448333	1.518644	23.55095

Source	DF	Type I SS	Mean Square	F Value	Pr > F
entry	85	2268.390439	26.686946	3.89	<.0001
rep	1	15.956850	15.956850	2.33	0.1306
entry*rep	85	583.270307	6.862004	2.98	<.0001

ANOVA Dependent Variable: Capsule diameter (2005 data only)

Number of Observations Read	855
Number of Observations Used	854

Class	Levels
entry	86
rep	2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	170	1650.825733	9.710740	22.40	<.0001
Error	683	296.128640	0.433570		
Corrected Total	853	1946.954373			

R-Square	Coeff Var	Root MSE	cap_mm Mean
0.847902	6.166485	0.658461	10.67806

Source	DF	Type I SS	Mean Square	F Value	Pr > F
entry	85	1464.562773	17.230150	7.84	<.0001
rep	1	1.683188	1.683188	0.77	0.3827
entry*rep	84	184.579772	2.197378	5.07	<.0001

ANOVA Dependent Variable: Days to flower (2005 data only)

Number of Observations Read	172
Number of Observations Used	172

Class	Levels
rep	2
entryna	86

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	86	27777.06977	322.98918	25.55	<.0001
Error	85	1074.62791	12.64268		
Corrected Total	171	28851.69767			

R-Square	Coeff Var	Root MSE	dayflow Mean
0.962753	8.032212	3.555655	44.26744

Source	DF	Type I SS	Mean Square	F Value	Pr > F
entryna	85	27776.69767	326.78468	25.85	<.0001
rep	1	0.37209	0.37209	0.03	0.8642

ANOVA Dependent Variable: 2006 Alkaloid data investigating effect of topping

Number of Observations Read	688
Number of Observations Used	686

Class	Levels
entry	86
rep	4
top	2
loc	2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	429	1043.908687	2.433354	3.77	<.0001
Error	256	165.167825	0.645187		
Corrected Total	685	1209.076512			

R-Square	Coeff Var	Root MSE	alk Mean
0.863393	35.40618	0.803235	2.268630

Source	DF	Type I SS	Mean Square	F Value	Pr > F
entry	85	171.9569834	2.0230233	1.06	0.3536
loc	1	7.8586934	7.8586934	13.68	0.0004
rep(loc)	2	1.3784559	0.6892280	1.19	0.3067
top	1	538.1266600	538.1266600	268.82	<.0001
entry*loc	85	49.1634148	0.5783931	0.90	0.7194
entry*rep	170	105.2680311	0.6192237	0.96	0.6116
entry*top	85	170.1564483	2.0018406	3.10	<.0001

Least Squares Means

top	alk LSMEAN	H0:LSMean1= LSMean2 Pr > |t|
post	3.15893555	<.0001
pre	1.38500000	

ANOVA Dependent Variable: Pre-topping PTA

Number of Observations Read	344
Number of Observations Used	344

Class	Levels
entry	86
rep	4
loc	2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	173	94.4466570	0.5459344	1.12	0.2349
Error	170	83.0875430	0.4887503		
Corrected Total	343	177.5342000			

R-Square	Coeff Var	Root MSE	alk Mean
0.531991	50.47702	0.699107	1.385000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
entry	85	37.21255000	0.43779471	0.80	0.8472
loc	1	5.89186163	5.89186163	10.72	0.0015
rep(loc)	2	4.62265698	2.31132849	4.73	0.0100
entry*loc	85	46.71958837	0.54964222	1.12	0.2583

ANOVA Dependent Variable: Post-topping PTA

Number of Observations Read	688
Number of Observations Used	513

Class	Levels
entry	86
year	2
rep	4
loc	2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	429	1455.342872	3.392408	4.30	<.0001
Error	83	65.418752	0.788178		
Corrected Total	512	1520.761624			

R-Square	Coeff Var	Root MSE	alk Mean
0.956983	22.66786	0.887794	3.916530

Source	DF	Type I SS	Mean Square	F Value	Pr > F
entry	85	559.6345438	6.5839358	4.79	<.0001
loc	1	118.9768985	118.9768985	108.27	<.0001
year	1	468.2852046	468.2852046	450.89	<.0001
rep(year)	3	6.2184509	2.0728170	2.91	0.0361
entry*loc	85	93.4075405	1.0989122	1.39	0.0651
entry*year	85	88.2787482	1.0385735	1.32	0.1044
entry*rep	169	120.5414856	0.7132632	0.90	0.7089

APPENDIX B

Calculated Distances between *N. rustica* Accessions

DGOWER MORPHOLOGICAL DISTANCES

ENTRY	TR1	TR10	TR11	TR12	TR13	TR14	TR15	TR16	TR17
TR1	0.00000	.	.	.	.	.	.	.	.
TR10	0.21164	0.00000	.	.	.	.	.	.	.
TR11	0.23948	0.07780	0.00000	.	.	.	.	.	.
TR12	0.22931	0.11647	0.13764	0.00000	.	.	.	.	.
TR13	0.33177	0.34530	0.34301	0.28869	0.00000	.	.	.	.
TR14	0.34583	0.36081	0.35852	0.29703	0.05468	0.00000	.	.	.
TR15	0.44712	0.47774	0.48199	0.42360	0.24709	0.24183	0.00000	.	.
TR16	0.45203	0.45474	0.48455	0.43358	0.31348	0.26677	0.17130	0.00000	.
TR17	0.43620	0.47099	0.45968	0.46204	0.25323	0.27733	0.24163	0.22011	0.00000
TR18	0.32886	0.40148	0.39671	0.32097	0.30790	0.31700	0.42125	0.48050	0.46982
TR2	0.22314	0.13044	0.13929	0.20343	0.38130	0.37113	0.49927	0.42871	0.45422
TR20	0.58110	0.52648	0.53794	0.51622	0.35317	0.33881	0.27844	0.22838	0.25734
TR21	0.54184	0.52006	0.52413	0.44884	0.30450	0.28789	0.21436	0.27038	0.26855
TR22	0.59503	0.53914	0.54528	0.48127	0.34018	0.32612	0.25969	0.29515	0.29602
TR23	0.35905	0.39122	0.38315	0.36283	0.38530	0.37304	0.24768	0.24490	0.35053
TR24	0.54096	0.53491	0.51235	0.46402	0.34955	0.37421	0.15368	0.30999	0.34173
TR25	0.43854	0.50756	0.50472	0.40846	0.38018	0.37797	0.32808	0.43959	0.46186
TR26	0.55490	0.55848	0.53220	0.49466	0.36055	0.37615	0.25716	0.38590	0.34993
TR27	0.57228	0.58666	0.56037	0.53086	0.38873	0.40970	0.26609	0.41945	0.37810
TR28	0.60505	0.55631	0.54923	0.48288	0.36769	0.35621	0.26130	0.34029	0.34559
TR29	0.49793	0.34020	0.32768	0.34954	0.38560	0.41605	0.36701	0.36761	0.34530
TR3	0.17308	0.16368	0.19571	0.21247	0.37700	0.37542	0.49234	0.45986	0.49367
TR30	0.37134	0.25552	0.25268	0.20634	0.30745	0.30460	0.35852	0.46182	0.45814
TR31	0.35494	0.35379	0.36712	0.32611	0.24464	0.21866	0.38955	0.38630	0.45377
TR32	0.52191	0.51482	0.48853	0.45918	0.32264	0.33113	0.13956	0.26171	0.31542
TR33	0.56735	0.54397	0.54020	0.44661	0.33804	0.34625	0.23667	0.34964	0.26868
TR34	0.53022	0.51454	0.51407	0.41777	0.31174	0.31995	0.21690	0.30901	0.24238
TR35	0.46675	0.48377	0.47909	0.42615	0.35171	0.34403	0.24356	0.33282	0.35598
TR36	0.44769	0.45311	0.46066	0.43844	0.43030	0.40761	0.30994	0.36273	0.39954
TR37	0.65894	0.65464	0.64757	0.54597	0.42978	0.42965	0.30833	0.43474	0.35761
TR38	0.60281	0.58396	0.55648	0.53913	0.47859	0.48532	0.30547	0.41059	0.45114
TR39	0.49684	0.48281	0.46737	0.43786	0.30227	0.35471	0.28322	0.38155	0.21753
TR4	0.31185	0.24342	0.24303	0.25979	0.28198	0.29378	0.35736	0.34472	0.33861
TR40	0.56625	0.51994	0.49524	0.47604	0.42157	0.43689	0.33409	0.44664	0.35221
TR41	0.54835	0.51909	0.46671	0.49045	0.43455	0.45042	0.34613	0.46933	0.34379
TR42	0.57568	0.55904	0.52871	0.51603	0.44239	0.46704	0.35330	0.47679	0.35764
TR43	0.57336	0.50348	0.50064	0.45037	0.34598	0.34799	0.26428	0.34178	0.25138
TR44	0.43644	0.43002	0.43995	0.39293	0.33558	0.33529	0.23325	0.33054	0.27067
TR45	0.43331	0.45154	0.45383	0.41131	0.25222	0.24558	0.13460	0.25710	0.15444
TR46	0.32748	0.37258	0.35716	0.36101	0.35649	0.35729	0.33520	0.41565	0.38999
TR47	0.44609	0.46603	0.45061	0.41096	0.23683	0.24998	0.17280	0.26783	0.22697
TR48	0.37483	0.42440	0.43333	0.36273	0.24940	0.22605	0.21640	0.20046	0.13931
TR49	0.30392	0.18243	0.20950	0.22011	0.37047	0.37820	0.34127	0.31873	0.40243

DGOWER MORPHOLOGICAL DISTANCES

ENTRY	TR1	TR10	TR11	TR12	TR13	TR14	TR15	TR16	TR17
TR5	0.14235	0.25110	0.25969	0.20663	0.30142	0.28399	0.37849	0.41496	0.44497
TR50	0.60025	0.60125	0.57814	0.63053	0.53575	0.53467	0.44782	0.46751	0.32618
TR51	0.54241	0.58080	0.55929	0.54580	0.43542	0.43756	0.32898	0.44518	0.33276
TR52	0.53363	0.51750	0.52045	0.47227	0.31493	0.30752	0.21613	0.30065	0.20670
TR53	0.52147	0.51680	0.49530	0.50114	0.32628	0.33812	0.24830	0.34306	0.22391
TR54	0.52195	0.44799	0.44238	0.39642	0.29080	0.27638	0.22403	0.23009	0.26769
TR55	0.39866	0.28419	0.27950	0.27028	0.26231	0.24535	0.41189	0.42380	0.45857
TR56	0.50661	0.52459	0.50650	0.46385	0.37703	0.38658	0.26339	0.39941	0.27471
TR57	0.45765	0.41454	0.41380	0.37327	0.26151	0.23578	0.12322	0.15586	0.24648
TR58	0.41279	0.45123	0.43061	0.41325	0.16235	0.18275	0.24663	0.37260	0.30532
TR59	0.44132	0.32690	0.31801	0.24965	0.36360	0.37551	0.31662	0.38705	0.38391
TR6	0.17345	0.18839	0.20497	0.11945	0.31460	0.31366	0.45326	0.45380	0.47776
TR60	0.28807	0.30045	0.31210	0.29572	0.34120	0.32893	0.27492	0.29028	0.38487
TR61	0.40455	0.41693	0.42471	0.42665	0.34273	0.32720	0.24823	0.29570	0.31843
TR62	0.46543	0.40619	0.41457	0.41460	0.19831	0.17399	0.27135	0.29710	0.32732
TR63	0.29385	0.17245	0.19578	0.11858	0.29605	0.26973	0.39307	0.41053	0.44761
TR64	0.34629	0.40500	0.41386	0.37887	0.31075	0.29839	0.24543	0.32406	0.30160
TR65	0.27431	0.26803	0.28468	0.23799	0.38010	0.36878	0.29542	0.34983	0.42725
TR66	0.33189	0.47434	0.48110	0.43704	0.34707	0.33992	0.25128	0.30757	0.31495
TR67	0.42712	0.48775	0.48829	0.41924	0.20530	0.19705	0.29595	0.30906	0.28337
TR68	0.39042	0.43333	0.42053	0.40228	0.25605	0.28398	0.24077	0.26674	0.15726
TR69	0.41952	0.44152	0.42784	0.38785	0.25005	0.26958	0.24268	0.26308	0.20754
TR7	0.13452	0.19995	0.22984	0.15818	0.29380	0.27202	0.39871	0.41122	0.44491
TR70	0.56088	0.50479	0.49430	0.51561	0.35837	0.37375	0.27083	0.33629	0.30924
TR71	0.43772	0.41564	0.40273	0.39794	0.24848	0.25814	0.20280	0.28609	0.22252
TR72	0.42207	0.34731	0.34906	0.35292	0.23998	0.27303	0.14358	0.21189	0.21610
TR73	0.38878	0.41366	0.40447	0.36527	0.21668	0.23555	0.19576	0.25811	0.18183
TR74	0.39050	0.40602	0.40319	0.38930	0.21473	0.23741	0.12707	0.14799	0.15668
TR75	0.41142	0.41418	0.41392	0.35203	0.12498	0.11033	0.27291	0.28876	0.26971
TR76	0.48355	0.48366	0.49593	0.42863	0.19442	0.19900	0.23720	0.36035	0.27610
TR77	0.53990	0.55788	0.52736	0.47975	0.33128	0.34294	0.20794	0.35023	0.30574
TR78	0.50362	0.41727	0.41760	0.35420	0.33410	0.31203	0.23571	0.31593	0.31404
TR79	0.48860	0.44656	0.45525	0.46626	0.37726	0.37394	0.31080	0.32559	0.23268
TR8	0.21683	0.14654	0.13945	0.13837	0.35665	0.35366	0.42776	0.50542	0.51462
TR80	0.53209	0.49628	0.49344	0.41962	0.33390	0.33639	0.23135	0.35476	0.26159
TR81	0.54895	0.56238	0.54696	0.50648	0.42192	0.44175	0.32092	0.45128	0.32001
TR82	0.27458	0.42505	0.39930	0.38483	0.28109	0.29257	0.31803	0.37123	0.39029
TR9	0.23845	0.18879	0.13864	0.15961	0.30644	0.32984	0.41622	0.49051	0.44959
TW116	0.51988	0.52414	0.46567	0.50815	0.43740	0.45230	0.40873	0.47924	0.33875
TW117	0.24453	0.38410	0.34025	0.34300	0.20001	0.18824	0.34438	0.38228	0.38340
TW118	0.58650	0.62826	0.58409	0.61613	0.51240	0.51808	0.43604	0.46963	0.32403
TW119	0.32127	0.43539	0.40965	0.38900	0.34940	0.39578	0.32759	0.41678	0.23639
TW120	0.35243	0.38120	0.33753	0.40655	0.40406	0.44149	0.39596	0.47460	0.30419

DGOWER MORPHOLOGICAL DISTANCES

ENTRY	TR18	TR2	TR20	TR21	TR22	TR23	TR24	TR25	TR26
TR1	.	.	.	.	.	.	.	.	.
TR10	.	.	.	.	.	.	.	.	.
TR11	.	.	.	.	.	.	.	.	.
TR12	.	.	.	.	.	.	.	.	.
TR13	.	.	.	.	.	.	.	.	.
TR14	.	.	.	.	.	.	.	.	.
TR15	.	.	.	.	.	.	.	.	.
TR16	.	.	.	.	.	.	.	.	.
TR17	.	.	.	.	.	.	.	.	.
TR18	0.00000	.	.	.	.	.	.	.	.
TR2	0.50281	0.00000	.	.	.	.	.	.	.
TR20	0.47506	0.49590	0.00000	.	.	.	.	.	.
TR21	0.40768	0.54472	0.08930	0.00000	.	.	.	.	.
TR22	0.39590	0.59559	0.15015	0.10846	0.00000	.	.	.	.
TR23	0.30767	0.41532	0.41528	0.37329	0.34545	0.00000	.	.	.
TR24	0.43968	0.57615	0.32025	0.26285	0.24316	0.26917	0.00000	.	.
TR25	0.40674	0.57135	0.39421	0.34106	0.29627	0.33032	0.28636	0.00000	.
TR26	0.41990	0.57400	0.26286	0.20098	0.15681	0.34888	0.18502	0.23972	0.00000
TR27	0.41044	0.59422	0.28653	0.21206	0.19477	0.37705	0.18103	0.30238	0.09961
TR28	0.41527	0.61276	0.22253	0.16140	0.11677	0.36788	0.19875	0.26968	0.14592
TR29	0.39242	0.34472	0.30100	0.30881	0.31227	0.30549	0.37729	0.43693	0.28120
TR3	0.34490	0.18291	0.53296	0.53467	0.53205	0.26353	0.49110	0.39897	0.49674
TR30	0.44271	0.31931	0.48707	0.41971	0.37262	0.38597	0.38603	0.27955	0.35443
TR31	0.27372	0.41025	0.47164	0.44698	0.39138	0.26275	0.43582	0.24980	0.41790
TR32	0.46384	0.52239	0.34268	0.25823	0.25202	0.22830	0.14412	0.30200	0.16045
TR33	0.46374	0.60042	0.27056	0.19695	0.22587	0.37419	0.17576	0.29893	0.24015
TR34	0.42704	0.57099	0.25605	0.19865	0.18753	0.34072	0.15980	0.25661	0.21426
TR35	0.32690	0.51734	0.36601	0.29863	0.25245	0.29947	0.23965	0.33385	0.17957
TR36	0.30814	0.51347	0.35154	0.31797	0.26625	0.20606	0.34033	0.24262	0.25065
TR37	0.51538	0.71110	0.38339	0.30892	0.27216	0.45683	0.22402	0.28196	0.22888
TR38	0.54474	0.59033	0.43823	0.36706	0.33284	0.29624	0.20150	0.28827	0.22802
TR39	0.47102	0.52167	0.34439	0.28699	0.28329	0.35262	0.20360	0.36649	0.22651
TR4	0.31619	0.27961	0.33016	0.31303	0.34503	0.37296	0.42781	0.53747	0.36815
TR40	0.51860	0.55630	0.36756	0.32026	0.34639	0.40501	0.28227	0.33199	0.32870
TR41	0.52058	0.50084	0.39161	0.32721	0.35559	0.35009	0.26830	0.36736	0.29646
TR42	0.55889	0.53303	0.38641	0.32204	0.36498	0.39300	0.30092	0.39183	0.31397
TR43	0.49653	0.56727	0.26054	0.21372	0.22248	0.36421	0.18575	0.29493	0.21870
TR44	0.40664	0.49233	0.40822	0.34084	0.34323	0.29696	0.32355	0.31723	0.32109
TR45	0.43603	0.47450	0.29262	0.22826	0.24886	0.30037	0.23337	0.32762	0.22451
TR46	0.29141	0.39873	0.34786	0.30458	0.28661	0.25414	0.37044	0.33096	0.28003
TR47	0.35563	0.44667	0.21895	0.15157	0.18754	0.27133	0.24614	0.37836	0.15024
TR48	0.41124	0.42082	0.29968	0.26457	0.31239	0.32337	0.33163	0.39617	0.32629
TR49	0.42685	0.21862	0.49175	0.47672	0.50015	0.26649	0.41875	0.53873	0.48349

DGOWER MORPHOLOGICAL DISTANCES

ENTRY	TR18	TR2	TR20	TR21	TR22	TR23	TR24	TR25	TR26
TR5	0.31824	0.23775	0.48965	0.45753	0.52891	0.32690	0.48509	0.38933	0.54149
TR50	0.67869	0.52805	0.39902	0.42157	0.48043	0.47235	0.42059	0.49993	0.43855
TR51	0.57632	0.54889	0.39504	0.32824	0.37128	0.41749	0.32627	0.37775	0.30037
TR52	0.51634	0.53608	0.22554	0.16244	0.23160	0.37864	0.21540	0.34764	0.26026
TR53	0.54929	0.51711	0.26045	0.20180	0.26296	0.36979	0.20484	0.35907	0.22246
TR54	0.33825	0.50445	0.19317	0.16436	0.12182	0.28088	0.24318	0.29824	0.16344
TR55	0.37074	0.29870	0.52047	0.47837	0.41697	0.31092	0.42390	0.36039	0.37455
TR56	0.44325	0.52482	0.38833	0.32094	0.31132	0.32008	0.32048	0.35829	0.30827
TR57	0.41414	0.46639	0.29150	0.24816	0.23799	0.21896	0.22355	0.33573	0.28904
TR58	0.25988	0.44799	0.33308	0.26944	0.29754	0.39303	0.31644	0.44343	0.23525
TR59	0.26409	0.40554	0.35716	0.29988	0.31690	0.32215	0.32727	0.36517	0.30407
TR6	0.24460	0.26913	0.52554	0.46826	0.47904	0.27854	0.47959	0.35161	0.51023
TR60	0.33263	0.35181	0.44750	0.41437	0.39810	0.14487	0.33633	0.37883	0.42466
TR61	0.30332	0.46445	0.25173	0.23142	0.24204	0.22550	0.34600	0.26075	0.25765
TR62	0.29147	0.44706	0.28683	0.26861	0.26807	0.36995	0.39175	0.44778	0.30393
TR63	0.39908	0.23624	0.50599	0.44410	0.43277	0.37257	0.48547	0.36004	0.47403
TR64	0.25554	0.44416	0.31118	0.24765	0.27145	0.22705	0.37371	0.28409	0.28930
TR65	0.41189	0.26295	0.54423	0.50070	0.53553	0.34358	0.41574	0.50803	0.50777
TR66	0.27656	0.46477	0.29908	0.26995	0.32426	0.26648	0.33929	0.31184	0.30114
TR67	0.30343	0.47099	0.25828	0.24530	0.32066	0.46284	0.37186	0.46224	0.33694
TR68	0.37352	0.43003	0.19678	0.18861	0.26000	0.35664	0.30459	0.44865	0.25831
TR69	0.34190	0.45885	0.17457	0.15480	0.22618	0.38411	0.31230	0.41563	0.26602
TR7	0.29829	0.21777	0.47256	0.41799	0.48842	0.32000	0.49624	0.36084	0.52689
TR70	0.45448	0.50501	0.19728	0.18289	0.14902	0.33132	0.21769	0.35510	0.12339
TR71	0.35859	0.45084	0.20847	0.14277	0.17241	0.27475	0.22122	0.35128	0.14598
TR72	0.40107	0.42721	0.29624	0.25496	0.28356	0.21782	0.19631	0.40483	0.30032
TR73	0.31344	0.43717	0.19958	0.15747	0.22885	0.32423	0.29608	0.43654	0.24980
TR74	0.40783	0.42972	0.26926	0.24893	0.28145	0.22968	0.23945	0.43259	0.32914
TR75	0.21807	0.44686	0.27886	0.22891	0.26053	0.37725	0.39574	0.47501	0.32116
TR76	0.34610	0.53944	0.36595	0.30267	0.30571	0.42687	0.28359	0.35806	0.30207
TR77	0.36821	0.55810	0.23589	0.17005	0.15481	0.32593	0.21060	0.31345	0.11022
TR78	0.37337	0.44703	0.22710	0.14714	0.20226	0.33548	0.32878	0.31876	0.25380
TR79	0.51258	0.45723	0.29771	0.30219	0.32848	0.34338	0.30502	0.37994	0.36156
TR8	0.37676	0.16294	0.57228	0.51500	0.53252	0.36589	0.43255	0.43957	0.48404
TR80	0.42625	0.56008	0.28733	0.23374	0.22858	0.34069	0.15929	0.24204	0.18847
TR81	0.51356	0.54759	0.34939	0.29337	0.32672	0.37614	0.27907	0.36999	0.30301
TR82	0.22495	0.38517	0.49666	0.45612	0.52301	0.24812	0.41328	0.48640	0.50328
TR9	0.38383	0.17438	0.53716	0.50327	0.56970	0.44252	0.47487	0.49580	0.54950
TW116	0.55631	0.46560	0.43221	0.40752	0.45407	0.40209	0.39525	0.45433	0.39613
TW117	0.30228	0.33025	0.44445	0.42180	0.49318	0.31562	0.43558	0.35386	0.47694
TW118	0.66429	0.57232	0.41381	0.41733	0.46059	0.50452	0.40958	0.47707	0.44110
TW119	0.30438	0.43094	0.38993	0.36378	0.41494	0.27094	0.36031	0.36701	0.39312
TW120	0.35383	0.42160	0.43896	0.39326	0.42358	0.28338	0.37731	0.36622	0.36874

DGOWER MORPHOLOGICAL DISTANCES

ENTRY	TR27	TR28	TR29	TR3	TR30	TR31	TR32	TR33	TR34
TR1	.	.	.	.	.	.	.	.	.
TR10	.	.	.	.	.	.	.	.	.
TR11	.	.	.	.	.	.	.	.	.
TR12	.	.	.	.	.	.	.	.	.
TR13	.	.	.	.	.	.	.	.	.
TR14	.	.	.	.	.	.	.	.	.
TR15	.	.	.	.	.	.	.	.	.
TR16	.	.	.	.	.	.	.	.	.
TR17	.	.	.	.	.	.	.	.	.
TR18	.	.	.	.	.	.	.	.	.
TR2	.	.	.	.	.	.	.	.	.
TR20	.	.	.	.	.	.	.	.	.
TR21	.	.	.	.	.	.	.	.	.
TR22	.	.	.	.	.	.	.	.	.
TR23	.	.	.	.	.	.	.	.	.
TR24	.	.	.	.	.	.	.	.	.
TR25	.	.	.	.	.	.	.	.	.
TR26	.	.	.	.	.	.	.	.	.
TR27	0.00000	.	.	.	.	.	.	.	.
TR28	0.17526	0.00000	.	.	.	.	.	.	.
TR29	0.28444	0.32743	0.00000	.	.	.	.	.	.
TR3	0.53482	0.52170	0.38243	0.00000	.	.	.	.	.
TR30	0.39907	0.35670	0.33512	0.30077	0.00000	.	.	.	.
TR31	0.46252	0.37627	0.38907	0.24225	0.24152	0.00000	.	.	.
TR32	0.16891	0.21799	0.30587	0.45591	0.31312	0.37096	0.00000	.	.
TR33	0.24041	0.17037	0.39016	0.53003	0.41793	0.44528	0.24691	0.00000	.
TR34	0.22839	0.18534	0.36644	0.50085	0.37922	0.40657	0.23812	0.06516	0.00000
TR35	0.15735	0.22928	0.30201	0.42187	0.36919	0.36039	0.22433	0.33770	0.29016
TR36	0.30195	0.23456	0.28330	0.34157	0.31410	0.22322	0.29730	0.39114	0.36179
TR37	0.27090	0.19278	0.49573	0.62003	0.42119	0.48099	0.29481	0.11492	0.15613
TR38	0.26259	0.26496	0.35681	0.51662	0.37461	0.43630	0.18240	0.31446	0.31852
TR39	0.25766	0.23491	0.30821	0.46247	0.43085	0.46426	0.24774	0.13249	0.12683
TR4	0.39588	0.36220	0.33458	0.28104	0.37047	0.41755	0.40096	0.42678	0.39735
TR40	0.35568	0.29188	0.40778	0.53282	0.42825	0.47021	0.34737	0.17943	0.19068
TR41	0.31052	0.30793	0.36449	0.48237	0.43733	0.47909	0.29337	0.20060	0.20872
TR42	0.31942	0.30683	0.36751	0.53237	0.45651	0.50089	0.30879	0.17631	0.23168
TR43	0.23974	0.16293	0.35727	0.48998	0.37598	0.40611	0.22552	0.08272	0.10104
TR44	0.32376	0.35245	0.33683	0.44545	0.34088	0.35611	0.27394	0.25439	0.21727
TR45	0.25805	0.25600	0.32973	0.44886	0.32829	0.37740	0.17992	0.17732	0.14019
TR46	0.31149	0.29161	0.28483	0.26553	0.25073	0.26716	0.31579	0.39492	0.36027
TR47	0.16621	0.21238	0.20868	0.44076	0.38235	0.40587	0.18418	0.25649	0.23019
TR48	0.35326	0.32559	0.36665	0.44409	0.41590	0.40501	0.28226	0.23643	0.19665
TR49	0.51120	0.51733	0.28486	0.27329	0.28687	0.39173	0.36244	0.52206	0.48493

DGOWER MORPHOLOGICAL DISTANCES

ENTRY	TR27	TR28	TR29	TR3	TR30	TR31	TR32	TR33	TR34
TR5	0.54390	0.55171	0.49651	0.18804	0.33353	0.31141	0.50851	0.47998	0.45815
TR50	0.40646	0.41396	0.43143	0.58729	0.56931	0.58829	0.41956	0.31907	0.36450
TR51	0.30695	0.30833	0.38854	0.56386	0.44299	0.49858	0.29527	0.21307	0.25640
TR52	0.27432	0.18845	0.38207	0.53211	0.41783	0.43978	0.25634	0.07192	0.11057
TR53	0.23774	0.21146	0.36049	0.50401	0.40888	0.46705	0.22019	0.11224	0.14083
TR54	0.20690	0.11532	0.23469	0.41338	0.33183	0.29544	0.21309	0.24400	0.19323
TR55	0.41454	0.41773	0.30167	0.29835	0.18539	0.16079	0.32989	0.47461	0.43326
TR56	0.29825	0.36683	0.31460	0.49129	0.39347	0.43195	0.27262	0.25874	0.22921
TR57	0.32041	0.24889	0.32812	0.42942	0.35353	0.35637	0.15858	0.28219	0.23711
TR58	0.24219	0.30757	0.30188	0.43470	0.39407	0.34695	0.28480	0.35564	0.31528
TR59	0.30847	0.30765	0.17604	0.41512	0.32688	0.38636	0.34801	0.33807	0.30478
TR6	0.54288	0.48489	0.44028	0.12170	0.28528	0.25120	0.46657	0.46191	0.43431
TR60	0.45283	0.41639	0.35848	0.21743	0.28063	0.23145	0.30407	0.42354	0.38690
TR61	0.28368	0.24639	0.24015	0.35756	0.34759	0.26400	0.28178	0.34417	0.31986
TR62	0.33747	0.27650	0.32830	0.42374	0.36870	0.25057	0.32949	0.38726	0.35013
TR63	0.50384	0.44175	0.35068	0.25781	0.15390	0.27576	0.41894	0.46719	0.42051
TR64	0.32285	0.27274	0.24493	0.34776	0.35408	0.28067	0.31487	0.37400	0.33687
TR65	0.49356	0.54411	0.41020	0.35472	0.30020	0.44784	0.38368	0.52320	0.48284
TR66	0.27499	0.33480	0.29425	0.41497	0.40193	0.33524	0.34508	0.36937	0.33103
TR67	0.34808	0.33838	0.39522	0.50390	0.45481	0.39035	0.39308	0.35498	0.31118
TR68	0.28648	0.27427	0.27048	0.46948	0.45588	0.47940	0.30632	0.31579	0.28950
TR69	0.29420	0.25152	0.29888	0.50610	0.47165	0.48191	0.33055	0.27613	0.25647
TR7	0.55506	0.50956	0.45583	0.14405	0.30995	0.28985	0.48492	0.46859	0.43245
TR70	0.13108	0.15068	0.19982	0.46454	0.40572	0.40706	0.20679	0.23293	0.21164
TR71	0.17415	0.18243	0.20995	0.40772	0.36383	0.39509	0.20467	0.24761	0.21048
TR72	0.32849	0.29622	0.27557	0.39534	0.38776	0.39970	0.17973	0.28388	0.25445
TR73	0.27797	0.27388	0.25344	0.45342	0.43431	0.44622	0.29062	0.29598	0.27738
TR74	0.35731	0.31631	0.32737	0.45215	0.42759	0.42251	0.21889	0.31176	0.28546
TR75	0.35430	0.29458	0.32806	0.44588	0.40068	0.31357	0.35442	0.36460	0.33830
TR76	0.30393	0.30700	0.44056	0.49844	0.35964	0.30417	0.30251	0.21646	0.17610
TR77	0.10803	0.16814	0.25101	0.49814	0.37873	0.41935	0.16878	0.23371	0.21154
TR78	0.27025	0.19802	0.22288	0.50880	0.29531	0.39876	0.29088	0.27949	0.28310
TR79	0.39271	0.31995	0.33915	0.46133	0.42938	0.41485	0.37474	0.23422	0.21256
TR8	0.48031	0.53499	0.37422	0.19711	0.22197	0.36180	0.45106	0.47863	0.44348
TR80	0.22253	0.20062	0.38374	0.46558	0.35182	0.39936	0.21918	0.11034	0.08656
TR81	0.30035	0.33035	0.34747	0.53404	0.45329	0.49518	0.32017	0.18319	0.20984
TR82	0.51164	0.57185	0.42831	0.34534	0.49956	0.34040	0.39231	0.50550	0.47829
TR9	0.52776	0.58126	0.40113	0.28893	0.27888	0.43335	0.51651	0.51993	0.48769
TW116	0.37098	0.45461	0.37867	0.51017	0.51204	0.55017	0.38318	0.34287	0.31678
TW117	0.49344	0.51369	0.44692	0.30831	0.36447	0.20410	0.44396	0.44553	0.42548
TW118	0.45250	0.42691	0.49523	0.63233	0.52890	0.58789	0.44969	0.32873	0.33275
TW119	0.39557	0.45247	0.35516	0.37064	0.49218	0.43878	0.38683	0.30382	0.28198
TW120	0.39341	0.42889	0.32681	0.34503	0.47749	0.43146	0.36260	0.32493	0.29550

DGOWER MORPHOLOGICAL DISTANCES

ENTRY	TR35	TR36	TR37	TR38	TR39	TR4	TR40	TR41	TR42
TR1	.	.	.	.	.	.	.	.	.
TR10	.	.	.	.	.	.	.	.	.
TR11	.	.	.	.	.	.	.	.	.
TR12	.	.	.	.	.	.	.	.	.
TR13	.	.	.	.	.	.	.	.	.
TR14	.	.	.	.	.	.	.	.	.
TR15	.	.	.	.	.	.	.	.	.
TR16	.	.	.	.	.	.	.	.	.
TR17	.	.	.	.	.	.	.	.	.
TR18	.	.	.	.	.	.	.	.	.
TR2	.	.	.	.	.	.	.	.	.
TR20	.	.	.	.	.	.	.	.	.
TR21	.	.	.	.	.	.	.	.	.
TR22	.	.	.	.	.	.	.	.	.
TR23	.	.	.	.	.	.	.	.	.
TR24	.	.	.	.	.	.	.	.	.
TR25	.	.	.	.	.	.	.	.	.
TR26	.	.	.	.	.	.	.	.	.
TR27	.	.	.	.	.	.	.	.	.
TR28	.	.	.	.	.	.	.	.	.
TR29	.	.	.	.	.	.	.	.	.
TR3	.	.	.	.	.	.	.	.	.
TR30	.	.	.	.	.	.	.	.	.
TR31	.	.	.	.	.	.	.	.	.
TR32	.	.	.	.	.	.	.	.	.
TR33	.	.	.	.	.	.	.	.	.
TR34	.	.	.	.	.	.	.	.	.
TR35	0.00000	.	.	.	.	.	.	.	.
TR36	0.21649	0.00000	.	.	.	.	.	.	.
TR37	0.34690	0.38729	0.00000	.	.	.	.	.	.
TR38	0.38096	0.33030	0.30124	0.00000	.	.	.	.	.
TR39	0.29738	0.41450	0.21927	0.31835	0.00000	.	.	.	.
TR4	0.31048	0.36515	0.53745	0.55378	0.37383	0.00000	.	.	.
TR40	0.42666	0.40441	0.27480	0.27273	0.19300	0.45383	0.00000	.	.
TR41	0.41523	0.38586	0.30554	0.20852	0.19879	0.46981	0.08860	0.00000	.
TR42	0.45076	0.43603	0.25823	0.22351	0.18916	0.50498	0.10531	0.07978	0.00000
TR43	0.31743	0.35166	0.17435	0.29575	0.12827	0.37954	0.15990	0.21284	0.20918
TR44	0.26548	0.30750	0.33524	0.29998	0.24688	0.39287	0.16914	0.20238	0.22157
TR45	0.23768	0.29782	0.25817	0.35076	0.16005	0.33174	0.23662	0.23494	0.26068
TR46	0.32584	0.19453	0.46622	0.38124	0.39431	0.26010	0.40310	0.37167	0.39749
TR47	0.18589	0.27301	0.35103	0.33336	0.21933	0.27304	0.34002	0.29458	0.29611
TR48	0.27784	0.35717	0.32435	0.44879	0.21344	0.27657	0.30281	0.32519	0.35710
TR49	0.40779	0.41412	0.61566	0.43835	0.42523	0.27043	0.49879	0.47499	0.50693

DGOWER MORPHOLOGICAL DISTANCES

ENTRY	TR35	TR36	TR37	TR38	TR39	TR4	TR40	TR41	TR42
TR5	0.45658	0.41744	0.58124	0.58940	0.50049	0.25897	0.47423	0.48543	0.50443
TR50	0.53716	0.49182	0.41243	0.31476	0.30761	0.54579	0.22033	0.19851	0.16993
TR51	0.43668	0.42913	0.26000	0.21100	0.20685	0.52684	0.15106	0.13307	0.06825
TR52	0.37341	0.38745	0.17502	0.31240	0.15472	0.38627	0.19119	0.20398	0.17779
TR53	0.34107	0.39761	0.20787	0.28778	0.11174	0.37732	0.18576	0.17570	0.15691
TR54	0.17284	0.18829	0.29431	0.33443	0.26876	0.26616	0.35000	0.35776	0.39478
TR55	0.33694	0.34206	0.52026	0.40576	0.42578	0.42000	0.48354	0.44574	0.47579
TR56	0.34074	0.36361	0.33730	0.25155	0.25297	0.49441	0.21234	0.17176	0.17856
TR57	0.25476	0.30692	0.36474	0.32122	0.27416	0.27866	0.35358	0.37691	0.41687
TR58	0.22654	0.35987	0.42368	0.43741	0.28362	0.25054	0.43261	0.39900	0.42321
TR59	0.22735	0.26660	0.41952	0.42978	0.32929	0.29174	0.34721	0.36665	0.40381
TR6	0.44008	0.36353	0.55834	0.54115	0.46055	0.25133	0.48498	0.47812	0.52103
TR60	0.38976	0.24878	0.51313	0.37202	0.41085	0.28518	0.43109	0.41548	0.45020
TR61	0.27205	0.11706	0.42501	0.36675	0.36456	0.29224	0.31719	0.34815	0.37572
TR62	0.26921	0.27918	0.46811	0.48942	0.38039	0.20427	0.44191	0.46220	0.49414
TR63	0.39182	0.37654	0.54973	0.50621	0.44420	0.28647	0.46103	0.48254	0.52375
TR64	0.24513	0.14953	0.45485	0.39446	0.33244	0.27310	0.35514	0.36724	0.39918
TR65	0.38046	0.46349	0.59130	0.48665	0.48056	0.29874	0.51103	0.52259	0.54992
TR66	0.24300	0.21373	0.44378	0.42989	0.36894	0.33739	0.37059	0.39842	0.40405
TR67	0.32214	0.41783	0.46411	0.53870	0.37073	0.26687	0.44236	0.46464	0.49197
TR68	0.26996	0.34245	0.42646	0.44523	0.25955	0.23749	0.38410	0.36588	0.38924
TR69	0.28966	0.36013	0.39106	0.46163	0.26727	0.23917	0.35094	0.36297	0.38109
TR7	0.47294	0.40119	0.58096	0.56582	0.46617	0.25132	0.47318	0.48204	0.50537
TR70	0.26018	0.27351	0.31452	0.25753	0.21836	0.33744	0.32329	0.27210	0.27801
TR71	0.16831	0.25355	0.34602	0.32844	0.18532	0.22698	0.30842	0.28536	0.32355
TR72	0.29303	0.33516	0.39455	0.32460	0.22464	0.24645	0.33330	0.32734	0.36729
TR73	0.22866	0.30926	0.40921	0.44844	0.25104	0.21782	0.37599	0.36942	0.38567
TR74	0.32544	0.36247	0.40526	0.35342	0.26909	0.28710	0.39529	0.38704	0.40920
TR75	0.28990	0.34563	0.44800	0.50203	0.34846	0.19330	0.45842	0.46641	0.48857
TR76	0.30887	0.42179	0.27901	0.43360	0.22666	0.35335	0.28691	0.33142	0.34633
TR77	0.18476	0.27009	0.29598	0.28427	0.25371	0.37385	0.36033	0.31492	0.32249
TR78	0.31327	0.25153	0.36289	0.34091	0.35629	0.36034	0.31454	0.31454	0.32760
TR79	0.43239	0.36000	0.34136	0.27844	0.22409	0.40872	0.14357	0.15066	0.17664
TR8	0.38309	0.45437	0.56776	0.53195	0.45003	0.29009	0.47411	0.44764	0.49357
TR80	0.25751	0.29951	0.15984	0.30315	0.16968	0.37761	0.20649	0.21682	0.26489
TR81	0.42892	0.43246	0.28801	0.22057	0.19879	0.52323	0.13166	0.11641	0.07622
TR82	0.46354	0.43657	0.60840	0.47321	0.43989	0.37446	0.52692	0.48029	0.49626
TR9	0.45696	0.52952	0.61230	0.59741	0.47645	0.27014	0.47932	0.46778	0.51635
TW116	0.43882	0.48555	0.45355	0.31892	0.26986	0.44464	0.18734	0.14884	0.19643
TW117	0.43869	0.37937	0.55730	0.52485	0.45090	0.38828	0.42410	0.41627	0.43988
TW118	0.52368	0.53340	0.39555	0.37550	0.29365	0.54876	0.16965	0.21727	0.19432
TW119	0.40245	0.37875	0.40511	0.44104	0.24315	0.39339	0.32508	0.28715	0.30471
TW120	0.43245	0.36684	0.43560	0.38762	0.22688	0.37341	0.29324	0.24399	0.27334

DGOWER MORPHOLOGICAL DISTANCES

ENTRY	TR43	TR44	TR45	TR46	TR47	TR48	TR49	TR5	TR50
TR1	.	.	.	.	.	.	.	.	.
TR10	.	.	.	.	.	.	.	.	.
TR11	.	.	.	.	.	.	.	.	.
TR12	.	.	.	.	.	.	.	.	.
TR13	.	.	.	.	.	.	.	.	.
TR14	.	.	.	.	.	.	.	.	.
TR15	.	.	.	.	.	.	.	.	.
TR16	.	.	.	.	.	.	.	.	.
TR17	.	.	.	.	.	.	.	.	.
TR18	.	.	.	.	.	.	.	.	.
TR2	.	.	.	.	.	.	.	.	.
TR20	.	.	.	.	.	.	.	.	.
TR21	.	.	.	.	.	.	.	.	.
TR22	.	.	.	.	.	.	.	.	.
TR23	.	.	.	.	.	.	.	.	.
TR24	.	.	.	.	.	.	.	.	.
TR25	.	.	.	.	.	.	.	.	.
TR26	.	.	.	.	.	.	.	.	.
TR27	.	.	.	.	.	.	.	.	.
TR28	.	.	.	.	.	.	.	.	.
TR29	.	.	.	.	.	.	.	.	.
TR3	.	.	.	.	.	.	.	.	.
TR30	.	.	.	.	.	.	.	.	.
TR31	.	.	.	.	.	.	.	.	.
TR32	.	.	.	.	.	.	.	.	.
TR33	.	.	.	.	.	.	.	.	.
TR34	.	.	.	.	.	.	.	.	.
TR35	.	.	.	.	.	.	.	.	.
TR36	.	.	.	.	.	.	.	.	.
TR37	.	.	.	.	.	.	.	.	.
TR38	.	.	.	.	.	.	.	.	.
TR39	.	.	.	.	.	.	.	.	.
TR4	.	.	.	.	.	.	.	.	.
TR40	.	.	.	.	.	.	.	.	.
TR41	.	.	.	.	.	.	.	.	.
TR42	.	.	.	.	.	.	.	.	.
TR43	0.00000	.	.	.	.	.	.	.	.
TR44	0.23621	0.00000	.	.	.	.	.	.	.
TR45	0.16355	0.15093	0.00000	.	.	.	.	.	.
TR46	0.37506	0.34403	0.31573	0.00000	.	.	.	.	.
TR47	0.26479	0.26365	0.15041	0.24997	0.00000	.	.	.	.
TR48	0.22015	0.19996	0.11414	0.40664	0.22633	0.00000	.	.	.
TR49	0.47488	0.38297	0.37833	0.30329	0.37776	0.36606	0.00000	.	.

DGOWER MORPHOLOGICAL DISTANCES

ENTRY	TR43	TR44	TR45	TR46	TR47	TR48	TR49	TR5	TR50
TR5	0.50844	0.39943	0.40759	0.30585	0.41193	0.35903	0.35339	0.00000	.
TR50	0.28786	0.30481	0.34058	0.49084	0.40426	0.36749	0.53314	0.57388	0.00000
TR51	0.22503	0.19752	0.23008	0.40155	0.30001	0.31225	0.48837	0.52901	0.16291
TR52	0.08316	0.26178	0.14618	0.37405	0.23737	0.19508	0.46927	0.46318	0.26403
TR53	0.08349	0.25575	0.14885	0.36746	0.22710	0.21124	0.44378	0.48754	0.23739
TR54	0.21844	0.27675	0.20278	0.24920	0.14117	0.23931	0.40901	0.47188	0.46696
TR55	0.42784	0.35095	0.37003	0.33045	0.37367	0.41835	0.27907	0.40312	0.55360
TR56	0.29002	0.12558	0.18457	0.35375	0.23199	0.29870	0.45590	0.46501	0.28399
TR57	0.24274	0.25193	0.17756	0.35300	0.21743	0.16575	0.30165	0.41402	0.45873
TR58	0.36280	0.32687	0.23801	0.27111	0.14789	0.27994	0.36788	0.40150	0.50816
TR59	0.35095	0.28606	0.31656	0.33725	0.25668	0.29448	0.31582	0.38376	0.52723
TR6	0.46942	0.42742	0.43952	0.28256	0.43630	0.39187	0.31632	0.13248	0.63734
TR60	0.40115	0.31981	0.32380	0.15237	0.33205	0.35749	0.17945	0.25120	0.51735
TR61	0.28994	0.24198	0.24196	0.15201	0.20821	0.28373	0.36407	0.35878	0.40121
TR62	0.34108	0.33816	0.25319	0.27434	0.21613	0.26857	0.35818	0.41966	0.51898
TR63	0.43542	0.34832	0.36087	0.31536	0.40560	0.32424	0.22335	0.26830	0.59634
TR64	0.33911	0.24704	0.22827	0.19962	0.19148	0.23276	0.34802	0.31762	0.45802
TR65	0.50543	0.34614	0.36810	0.36926	0.42033	0.32666	0.15397	0.26880	0.54586
TR66	0.37206	0.25464	0.26377	0.23577	0.21335	0.24938	0.41207	0.28838	0.44074
TR67	0.37116	0.38659	0.29246	0.36044	0.23711	0.25189	0.44801	0.37916	0.48213
TR68	0.30792	0.33998	0.21656	0.33351	0.14249	0.19210	0.39205	0.39753	0.38964
TR69	0.29408	0.35045	0.23574	0.36191	0.15300	0.18825	0.42483	0.38200	0.42880
TR7	0.47519	0.39821	0.38908	0.28227	0.40025	0.33402	0.32005	0.10313	0.58702
TR70	0.20436	0.34844	0.24538	0.25546	0.13381	0.33772	0.43610	0.54370	0.33824
TR71	0.21575	0.25329	0.15933	0.24254	0.07594	0.20946	0.35306	0.41963	0.39701
TR72	0.24690	0.27721	0.19196	0.34970	0.20130	0.20805	0.25706	0.40140	0.43354
TR73	0.30752	0.29507	0.18970	0.32049	0.11507	0.18687	0.36783	0.36404	0.44824
TR74	0.29446	0.30930	0.20197	0.37695	0.19745	0.17486	0.27085	0.38718	0.42510
TR75	0.36471	0.36091	0.27321	0.29708	0.19624	0.22649	0.35965	0.37736	0.55556
TR76	0.21678	0.22895	0.16430	0.36058	0.27214	0.22863	0.43156	0.42281	0.45081
TR77	0.26272	0.30571	0.21785	0.26760	0.11143	0.33199	0.48919	0.50117	0.43622
TR78	0.29547	0.28539	0.24945	0.26183	0.18615	0.30267	0.35927	0.43750	0.41328
TR79	0.19659	0.17053	0.20855	0.38143	0.30261	0.23756	0.40057	0.43364	0.17739
TR8	0.49391	0.39038	0.41108	0.35075	0.42102	0.42505	0.26223	0.20398	0.61126
TR80	0.11468	0.23075	0.13940	0.37713	0.24768	0.20191	0.48171	0.45604	0.36657
TR81	0.20887	0.19843	0.24995	0.39178	0.27393	0.34641	0.49624	0.49554	0.20540
TR82	0.53403	0.40966	0.40768	0.36790	0.37061	0.36570	0.32436	0.26815	0.54836
TR9	0.53798	0.41762	0.42506	0.39126	0.43118	0.40076	0.27027	0.18566	0.59687
TW116	0.30004	0.22142	0.28816	0.47058	0.32583	0.29510	0.47960	0.46532	0.18268
TW117	0.47041	0.35737	0.35493	0.31823	0.35271	0.34209	0.41294	0.17527	0.49282
TW118	0.27819	0.27657	0.32619	0.50960	0.42962	0.35310	0.57341	0.54473	0.17403
TW119	0.33227	0.27113	0.26146	0.35538	0.29337	0.25923	0.41835	0.30819	0.37431
TW120	0.29815	0.29454	0.27976	0.36386	0.31465	0.29143	0.42159	0.36658	0.35501

DGOWER MORPHOLOGICAL DISTANCES

ENTRY	TR51	TR52	TR53	TR54	TR55	TR56	TR57	TR58	TR59
TR50	.	.	.	.	.	.	.	.	.
TR51	0.00000	.	.	.	.	.	.	.	.
TR52	0.17633	0.00000	.	.	.	.	.	.	.
TR53	0.14952	0.07191	0.00000	.	.	.	.	.	.
TR54	0.39235	0.25331	0.28391	0.00000	.	.	.	.	.
TR55	0.46228	0.47092	0.44175	0.34632	0.00000	.	.	.	.
TR56	0.18005	0.28748	0.27390	0.32425	0.40304	0.00000	.	.	.
TR57	0.38196	0.26055	0.26984	0.17496	0.37988	0.32835	0.00000	.	.
TR58	0.39336	0.34310	0.31366	0.23624	0.30901	0.33443	0.27454	0.00000	.
TR59	0.44982	0.39096	0.40203	0.22455	0.32843	0.35655	0.30796	0.29464	0.00000
TR6	0.57904	0.49320	0.51893	0.40294	0.34600	0.48682	0.39871	0.44773	0.32404
TR60	0.46258	0.40692	0.42112	0.31568	0.32354	0.38048	0.25377	0.36120	0.36651
TR61	0.34324	0.29977	0.30836	0.16879	0.36815	0.31929	0.23245	0.28376	0.25424
TR62	0.45927	0.33696	0.34823	0.18197	0.30014	0.41963	0.20968	0.13435	0.34152
TR63	0.51023	0.45919	0.47534	0.36543	0.23101	0.43667	0.29544	0.38393	0.29245
TR64	0.36342	0.31858	0.33686	0.20343	0.38606	0.32467	0.20864	0.24290	0.22403
TR65	0.49793	0.49248	0.48556	0.46750	0.34728	0.45320	0.30770	0.35755	0.33412
TR66	0.36558	0.33866	0.34352	0.25826	0.41378	0.34079	0.30799	0.27308	0.23087
TR67	0.47831	0.34304	0.35992	0.25196	0.41042	0.44061	0.28981	0.13438	0.31636
TR68	0.39499	0.26266	0.26644	0.20848	0.48020	0.36404	0.24387	0.21265	0.25258
TR69	0.40271	0.25238	0.28284	0.19199	0.50860	0.37419	0.24007	0.23236	0.21953
TR7	0.52020	0.43780	0.47967	0.42273	0.38687	0.47840	0.37204	0.40211	0.38625
TR70	0.28275	0.21658	0.19462	0.15110	0.39872	0.29237	0.28176	0.25456	0.33480
TR71	0.31901	0.21587	0.19180	0.12302	0.37142	0.27924	0.17024	0.14840	0.23238
TR72	0.39453	0.25240	0.25362	0.21936	0.40491	0.33984	0.09444	0.26323	0.25977
TR73	0.38804	0.26227	0.26965	0.19405	0.45063	0.32358	0.22881	0.19540	0.21168
TR74	0.38890	0.24978	0.27547	0.22982	0.43328	0.34312	0.12183	0.28030	0.32535
TR75	0.46775	0.33202	0.35484	0.21475	0.34026	0.40591	0.23907	0.11291	0.28867
TR76	0.32526	0.21838	0.23109	0.27506	0.32768	0.27868	0.26232	0.16837	0.37630
TR77	0.32398	0.27756	0.26399	0.15860	0.39108	0.20134	0.28407	0.21675	0.28849
TR78	0.30877	0.24677	0.27996	0.19801	0.35270	0.28591	0.26499	0.28741	0.19835
TR79	0.15868	0.16635	0.18552	0.29763	0.44309	0.21426	0.30389	0.39004	0.37875
TR8	0.54065	0.51775	0.49286	0.45189	0.26098	0.44647	0.42409	0.39353	0.27311
TR80	0.29235	0.15836	0.16763	0.20159	0.42203	0.25312	0.24768	0.32260	0.29173
TR81	0.10423	0.20129	0.17460	0.38907	0.47008	0.10732	0.40986	0.39676	0.40706
TR82	0.48974	0.48332	0.47408	0.49202	0.38376	0.39969	0.35511	0.28159	0.41867
TR9	0.54197	0.50490	0.49555	0.50143	0.34968	0.48425	0.44282	0.39157	0.31574
TW116	0.21074	0.31112	0.25702	0.42883	0.50461	0.21641	0.36940	0.40981	0.41574
TW117	0.46446	0.42515	0.42299	0.43600	0.27588	0.40971	0.41231	0.29345	0.37962
TW118	0.19284	0.28244	0.24157	0.48167	0.57649	0.32588	0.46566	0.51948	0.49290
TW119	0.32024	0.30849	0.27719	0.39448	0.48796	0.23828	0.35143	0.33698	0.33261
TW120	0.29868	0.30663	0.26515	0.38705	0.46881	0.28310	0.34117	0.38209	0.33719

DGOWER MORPHOLOGICAL DISTANCES

ENTRY	TR6	TR60	TR61	TR62	TR63	TR64	TR65	TR66	TR67
TR50	.	.	.	.	.	.	.	.	.
TR51	.	.	.	.	.	.	.	.	.
TR52	.	.	.	.	.	.	.	.	.
TR53	.	.	.	.	.	.	.	.	.
TR54	.	.	.	.	.	.	.	.	.
TR55	.	.	.	.	.	.	.	.	.
TR56	.	.	.	.	.	.	.	.	.
TR57	.	.	.	.	.	.	.	.	.
TR58	.	.	.	.	.	.	.	.	.
TR59	.	.	.	.	.	.	.	.	.
TR6	0.00000	.	.	.	.	.	.	.	.
TR60	0.23093	0.00000	.	.	.	.	.	.	.
TR61	0.37065	0.23447	0.00000	.	.	.	.	.	.
TR62	0.43552	0.31860	0.20807	0.00000	.	.	.	.	.
TR63	0.21439	0.26052	0.36095	0.31942	0.00000	.	.	.	.
TR64	0.32287	0.25665	0.08592	0.21006	0.30148	0.00000	.	.	.
TR65	0.34775	0.24492	0.40880	0.39511	0.21926	0.35631	0.00000	.	.
TR66	0.39460	0.28743	0.14053	0.30073	0.41624	0.13013	0.35151	0.00000	.
TR67	0.45382	0.41491	0.32798	0.17196	0.41539	0.29587	0.39783	0.26469	0.00000
TR68	0.41614	0.40180	0.26715	0.25952	0.43442	0.20986	0.40312	0.22407	0.16623
TR69	0.39204	0.42479	0.27231	0.27057	0.43061	0.22568	0.42776	0.23438	0.17989
TR7	0.09790	0.23495	0.34059	0.39957	0.20684	0.27865	0.30016	0.33479	0.38261
TR70	0.52156	0.37574	0.22287	0.26746	0.47560	0.28855	0.53512	0.31345	0.34513
TR71	0.41887	0.32225	0.18647	0.19546	0.36552	0.16246	0.40686	0.21895	0.21295
TR72	0.35765	0.25344	0.27112	0.27162	0.36219	0.23580	0.33383	0.31791	0.30687
TR73	0.38932	0.36862	0.23622	0.23853	0.41074	0.18929	0.37755	0.20506	0.20410
TR74	0.40501	0.27254	0.28717	0.28188	0.39439	0.24838	0.30489	0.28563	0.28651
TR75	0.36774	0.34243	0.26876	0.10354	0.33663	0.21842	0.37417	0.29060	0.14025
TR76	0.46311	0.36777	0.34533	0.18969	0.37618	0.33302	0.39814	0.34436	0.22104
TR77	0.48704	0.41127	0.26069	0.29842	0.46907	0.28038	0.48937	0.28907	0.31918
TR78	0.44684	0.36829	0.16950	0.27536	0.31241	0.18346	0.36575	0.24543	0.31546
TR79	0.48719	0.36061	0.27300	0.35440	0.44305	0.29704	0.44663	0.31139	0.38326
TR8	0.20301	0.30893	0.45492	0.44552	0.17781	0.41997	0.23107	0.41830	0.44535
TR80	0.42468	0.40061	0.31277	0.34691	0.43267	0.33365	0.48347	0.35168	0.32149
TR81	0.52957	0.44449	0.36871	0.48548	0.51803	0.38849	0.52309	0.37723	0.46739
TR82	0.32903	0.26502	0.37893	0.36288	0.43405	0.32066	0.27351	0.30797	0.32429
TR9	0.24138	0.35804	0.46540	0.45260	0.21931	0.40820	0.22022	0.38879	0.42155
TW116	0.51252	0.47733	0.39300	0.46475	0.48857	0.36798	0.45463	0.40007	0.45537
TW117	0.27389	0.28657	0.32076	0.32814	0.35856	0.31100	0.37767	0.24320	0.27043
TW118	0.61664	0.54196	0.43474	0.54599	0.59269	0.45274	0.53146	0.40697	0.45317
TW119	0.33413	0.36118	0.30345	0.44573	0.45514	0.25531	0.39836	0.25590	0.39208
TW120	0.32004	0.36286	0.30244	0.44897	0.44682	0.25855	0.45771	0.32965	0.45851

DGOWER MORPHOLOGICAL DISTANCES

ENTRY	TR68	TR69	TR7	TR70	TR71	TR72	TR73	TR74	TR75
TR50	.	.	.	.	.	.	.	.	.
TR51	.	.	.	.	.	.	.	.	.
TR52	.	.	.	.	.	.	.	.	.
TR53	.	.	.	.	.	.	.	.	.
TR54	.	.	.	.	.	.	.	.	.
TR55	.	.	.	.	.	.	.	.	.
TR56	.	.	.	.	.	.	.	.	.
TR57	.	.	.	.	.	.	.	.	.
TR58	.	.	.	.	.	.	.	.	.
TR59	.	.	.	.	.	.	.	.	.
TR6	.	.	.	.	.	.	.	.	.
TR60	.	.	.	.	.	.	.	.	.
TR61	.	.	.	.	.	.	.	.	.
TR62	.	.	.	.	.	.	.	.	.
TR63	.	.	.	.	.	.	.	.	.
TR64	.	.	.	.	.	.	.	.	.
TR65	.	.	.	.	.	.	.	.	.
TR66	.	.	.	.	.	.	.	.	.
TR67	.	.	.	.	.	.	.	.	.
TR68	0.00000	.	.	.	.	.	.	.	.
TR69	0.07213	0.00000	.	.	.	.	.	.	.
TR7	0.38952	0.37985	0.00000	.	.	.	.	.	.
TR70	0.24567	0.26207	0.52011	0.00000	.	.	.	.	.
TR71	0.11947	0.13987	0.39447	0.13507	0.00000	.	.	.	.
TR72	0.16811	0.18768	0.36881	0.27010	0.15434	0.00000	.	.	.
TR73	0.07565	0.08228	0.36343	0.24888	0.12268	0.16082	0.00000	.	.
TR74	0.14774	0.16290	0.38713	0.29166	0.19061	0.07676	0.13686	0.00000	.
TR75	0.19622	0.18544	0.35096	0.31354	0.19415	0.24996	0.15247	0.22235	0.00000
TR76	0.33982	0.32101	0.42802	0.32822	0.24905	0.30071	0.32770	0.32375	0.22230
TR77	0.24227	0.24122	0.51168	0.11729	0.15027	0.29620	0.20301	0.29918	0.28535
TR78	0.24551	0.21640	0.41285	0.24644	0.18665	0.30601	0.22970	0.28626	0.25146
TR79	0.30368	0.30481	0.43141	0.28480	0.26542	0.28975	0.30647	0.27833	0.39600
TR8	0.45718	0.47076	0.20492	0.50165	0.39279	0.39783	0.42939	0.46532	0.43962
TR80	0.28482	0.25809	0.44319	0.25605	0.20937	0.23971	0.27836	0.29601	0.35150
TR81	0.38561	0.38385	0.50117	0.25956	0.31654	0.37432	0.34635	0.38580	0.45931
TR82	0.37390	0.39268	0.28293	0.50442	0.40209	0.32078	0.32183	0.27830	0.27999
TR9	0.39214	0.40572	0.21617	0.55170	0.42550	0.38722	0.37184	0.40160	0.39543
TW116	0.35264	0.35926	0.48281	0.35722	0.30892	0.34918	0.37319	0.37744	0.46146
TW117	0.34788	0.35395	0.23338	0.47915	0.37577	0.38087	0.33248	0.36398	0.29361
TW118	0.36770	0.40462	0.57262	0.41910	0.39858	0.43831	0.43384	0.43598	0.54702
TW119	0.26361	0.28128	0.32970	0.39533	0.29376	0.28870	0.26180	0.28007	0.37320
TW120	0.30725	0.30256	0.34455	0.35376	0.27730	0.27244	0.32397	0.32661	0.42035

DGOWER MORPHOLOGICAL DISTANCES

ENTRY	TR76	TR77	TR78	TR79	TR8	TR80	TR81	TR82	TR9
TR50	.	.	.	.	.	.	.	.	.
TR51	.	.	.	.	.	.	.	.	.
TR52	.	.	.	.	.	.	.	.	.
TR53	.	.	.	.	.	.	.	.	.
TR54	.	.	.	.	.	.	.	.	.
TR55	.	.	.	.	.	.	.	.	.
TR56	.	.	.	.	.	.	.	.	.
TR57	.	.	.	.	.	.	.	.	.
TR58	.	.	.	.	.	.	.	.	.
TR59	.	.	.	.	.	.	.	.	.
TR6	.	.	.	.	.	.	.	.	.
TR60	.	.	.	.	.	.	.	.	.
TR61	.	.	.	.	.	.	.	.	.
TR62	.	.	.	.	.	.	.	.	.
TR63	.	.	.	.	.	.	.	.	.
TR64	.	.	.	.	.	.	.	.	.
TR65	.	.	.	.	.	.	.	.	.
TR66	.	.	.	.	.	.	.	.	.
TR67	.	.	.	.	.	.	.	.	.
TR68	.	.	.	.	.	.	.	.	.
TR69	.	.	.	.	.	.	.	.	.
TR7	.	.	.	.	.	.	.	.	.
TR70	.	.	.	.	.	.	.	.	.
TR71	.	.	.	.	.	.	.	.	.
TR72	.	.	.	.	.	.	.	.	.
TR73	.	.	.	.	.	.	.	.	.
TR74	.	.	.	.	.	.	.	.	.
TR75	.	.	.	.	.	.	.	.	.
TR76	0.00000	.	.	.	.	.	.	.	.
TR77	0.29036	0.00000	.	.	.	.	.	.	.
TR78	0.32780	0.21169	0.00000	.	.	.	.	.	.
TR79	0.29180	0.38410	0.29125	0.00000	.	.	.	.	.
TR8	0.42485	0.46645	0.41294	0.48813	0.00000	.	.	.	.
TR80	0.19280	0.21988	0.29400	0.24485	0.42936	0.00000	.	.	.
TR81	0.32318	0.25371	0.31979	0.16595	0.49698	0.25138	0.00000	.	.
TR82	0.37304	0.43661	0.45076	0.44926	0.39016	0.49037	0.43607	0.00000	.
TR9	0.44543	0.52042	0.40136	0.47605	0.13549	0.48558	0.51478	0.34861	0.00000
TW116	0.36932	0.38625	0.39111	0.19541	0.47542	0.31549	0.19705	0.45477	0.42896
TW117	0.32408	0.44300	0.40177	0.39364	0.31555	0.42735	0.43328	0.20141	0.28267
TW118	0.42301	0.48263	0.42800	0.20360	0.58765	0.36500	0.23137	0.56686	0.55442
TW119	0.32360	0.34025	0.34270	0.28190	0.40848	0.28854	0.25952	0.23837	0.36424
TW120	0.35491	0.37209	0.37057	0.27065	0.40796	0.28085	0.29106	0.31596	0.41269

DGOWER MORPHOLOGICAL DISTANCES

ENTRY	TW116	TW117	TW118	TW119	TW120
TR50	.	.	.	.	.
TR51	.	.	.	.	.
TR52	.	.	.	.	.
TR53	.	.	.	.	.
TR54	.	.	.	.	.
TR55	.	.	.	.	.
TR56	.	.	.	.	.
TR57	.	.	.	.	.
TR58	.	.	.	.	.
TR59	.	.	.	.	.
TR6	.	.	.	.	.
TR60	.	.	.	.	.
TR61	.	.	.	.	.
TR62	.	.	.	.	.
TR63	.	.	.	.	.
TR64	.	.	.	.	.
TR65	.	.	.	.	.
TR66	.	.	.	.	.
TR67	.	.	.	.	.
TR68	.	.	.	.	.
TR69	.	.	.	.	.
TR7	.	.	.	.	.
TR70	.	.	.	.	.
TR71	.	.	.	.	.
TR72	.	.	.	.	.
TR73	.	.	.	.	.
TR74	.	.	.	.	.
TR75	.	.	.	.	.
TR76	.	.	.	.	.
TR77	.	.	.	.	.
TR78	.	.	.	.	.
TR79	.	.	.	.	.
TR8	.	.	.	.	.
TR80	.	.	.	.	.
TR81	.	.	.	.	.
TR82	.	.	.	.	.
TR9	.	.	.	.	.
TW116	0.00000	.	.	.	.
TW117	0.42965	0.00000	.	.	.
TW118	0.21923	0.44639	0.00000	.	.
TW119	0.26541	0.31790	0.38251	0.00000	.
TW120	0.23512	0.36941	0.37367	0.11497	0.00000

JACCARD GENETIC DISTANCES

ENTRY	TR1	TR2	TR3	TR4	TR5	TR6	TR7	TR8	TR9
TR1	0.00000	.	.	.	.	.	.	.	.
TR2	0.16327	0.00000	.	.	.	.	.	.	.
TR3	0.11905	0.26531	0.00000	.	.	.	.	.	.
TR4	0.15909	0.26000	0.27273	0.00000	.	.	.	.	.
TR5	0.18182	0.24490	0.29545	0.02564	0.00000	.	.	.	.
TR6	0.14286	0.28571	0.26190	0.07692	0.10256	0.00000	.	.	.
TR7	0.23810	0.36735	0.18421	0.17949	0.20513	0.16216	0.00000	.	.
TR8	0.22222	0.28000	0.33333	0.12195	0.10000	0.15000	0.25000	0.00000	.
TR9	0.29545	0.41176	0.20513	0.24390	0.26829	0.27500	0.14286	0.30952	0.00000
TR10	0.23256	0.29167	0.34884	0.21951	0.20000	0.15789	0.30769	0.15385	0.32500
TR11	0.18605	0.32000	0.26190	0.07692	0.10256	0.05405	0.16216	0.15000	0.23077
TR12	0.26190	0.38776	0.34146	0.20513	0.23077	0.13889	0.25000	0.23077	0.35897
TR13	0.27907	0.40000	0.18421	0.22500	0.25000	0.25641	0.11765	0.29268	0.08824
TR14	0.23913	0.26000	0.34783	0.14286	0.12195	0.21429	0.30952	0.12195	0.32558
TR15	0.25581	0.38000	0.20513	0.20000	0.22500	0.23077	0.08824	0.26829	0.11429
TR16	0.26667	0.38462	0.30233	0.21429	0.23810	0.24390	0.25641	0.27907	0.23077
TR17	0.25000	0.37255	0.20000	0.19512	0.21951	0.22500	0.13889	0.26190	0.16216
TR18	0.34884	0.46000	0.26316	0.30000	0.32500	0.33333	0.20588	0.36585	0.12121
TR20	0.16279	0.30000	0.23810	0.10000	0.12500	0.12821	0.18421	0.17073	0.20513
TR21	0.27907	0.40000	0.18421	0.22500	0.25000	0.25641	0.11765	0.29268	0.08824
TR22	0.34783	0.45283	0.26829	0.30233	0.32558	0.33333	0.21622	0.32558	0.13889
TR23	0.33333	0.44231	0.25000	0.28571	0.30952	0.31707	0.19444	0.34884	0.16667
TR24	0.25000	0.37255	0.28571	0.15000	0.17500	0.17949	0.18919	0.21951	0.21053
TR25	0.39130	0.49057	0.31707	0.34884	0.37209	0.38095	0.27027	0.40909	0.19444
TR26	0.36957	0.47170	0.37209	0.28571	0.30952	0.31707	0.24324	0.34884	0.26316
TR27	0.23913	0.35849	0.27273	0.18605	0.20930	0.21429	0.22500	0.25000	0.20000
TR28	0.38298	0.48148	0.38636	0.30233	0.32558	0.33333	0.30769	0.32558	0.28205
TR29	0.41304	0.50943	0.34146	0.37209	0.39535	0.40476	0.29730	0.43182	0.22222
TR30	0.31111	0.42308	0.34884	0.26190	0.28571	0.29268	0.30769	0.32558	0.28205
TR31	0.34043	0.44444	0.30233	0.29545	0.31818	0.32558	0.21053	0.31818	0.23077
TR32	0.42553	0.51852	0.35714	0.38636	0.40909	0.41860	0.31579	0.44444	0.24324
TR33	0.35417	0.45455	0.39130	0.27273	0.29545	0.30233	0.31707	0.29545	0.33333
TR34	0.27660	0.38889	0.31111	0.22727	0.25000	0.25581	0.26829	0.28889	0.24390
TR35	0.32609	0.43396	0.32558	0.27907	0.30233	0.30952	0.28205	0.34091	0.30000
TR36	0.35417	0.45455	0.35556	0.27273	0.29545	0.30233	0.23077	0.33333	0.29268
TR37	0.39583	0.49091	0.32558	0.31818	0.34091	0.34884	0.23684	0.34091	0.21053
TR38	0.36957	0.47170	0.37209	0.32558	0.34884	0.35714	0.28947	0.38636	0.30769
TR41	0.30435	0.41509	0.34091	0.21429	0.23810	0.24390	0.25641	0.23810	0.31707
TR39	0.42857	0.51786	0.36364	0.39130	0.41304	0.42222	0.32500	0.44681	0.25641
TR40	0.24444	0.36538	0.27907	0.19048	0.21429	0.21951	0.23077	0.21429	0.25000
TR42	0.35556	0.46154	0.35714	0.30952	0.33333	0.30000	0.22222	0.37209	0.28947
TR43	0.34694	0.44643	0.38298	0.30435	0.32609	0.33333	0.34884	0.36170	0.32558
TR44	0.31818	0.43137	0.27500	0.22500	0.25000	0.25641	0.11765	0.29268	0.19444

JACCARD GENETIC DISTANCES

ENTRY	TR1	TR2	TR3	TR4	TR5	TR6	TR7	TR8	TR9
TR45	0.36170	0.46296	0.28571	0.31818	0.34091	0.34884	0.23684	0.34091	0.21053
TR46	0.22727	0.35294	0.30233	0.17073	0.19512	0.20000	0.25641	0.19512	0.23077
TR47	0.33333	0.44231	0.25000	0.28571	0.30952	0.31707	0.19444	0.30952	0.11429
TR48	0.32609	0.43396	0.28571	0.27907	0.30233	0.30952	0.23684	0.30233	0.21053
TR49	0.20000	0.32692	0.31111	0.14286	0.16667	0.17073	0.26829	0.16667	0.24390
TR50	0.28889	0.40385	0.20000	0.35556	0.37778	0.38636	0.28205	0.37778	0.30000
TR51	0.43750	0.52727	0.37209	0.36364	0.38636	0.39535	0.28947	0.42222	0.30769
TR52	0.41304	0.50943	0.34146	0.37209	0.39535	0.40476	0.29730	0.43182	0.17143
TR53	0.38298	0.48148	0.38636	0.30233	0.32558	0.33333	0.30769	0.36364	0.36585
TR54	0.28889	0.40385	0.32558	0.19512	0.21951	0.22500	0.18919	0.26190	0.25641
TR55	0.31111	0.42308	0.26829	0.26190	0.28571	0.29268	0.16667	0.28571	0.18919
TR56	0.33333	0.44231	0.25000	0.28571	0.30952	0.31707	0.24324	0.30952	0.16667
TR57	0.38298	0.48148	0.34884	0.34091	0.36364	0.37209	0.26316	0.36364	0.23684
TR58	0.35556	0.46154	0.31707	0.30952	0.33333	0.34146	0.22222	0.33333	0.19444
TR59	0.30435	0.41509	0.37778	0.25581	0.27907	0.28571	0.34146	0.27907	0.31707
TR60	0.32609	0.43396	0.36364	0.27907	0.30233	0.30952	0.28205	0.30233	0.25641
TR61	0.34043	0.41509	0.44681	0.25581	0.27907	0.32558	0.41860	0.31818	0.39535
TR62	0.31915	0.39623	0.35556	0.27273	0.29545	0.30233	0.31707	0.25581	0.29268
TR63	0.45652	0.54717	0.39024	0.38095	0.40476	0.41463	0.30556	0.40476	0.27778
TR64	0.30435	0.41509	0.34091	0.25581	0.27907	0.28571	0.30000	0.27907	0.27500
TR65	0.31915	0.39623	0.42553	0.23256	0.25581	0.30233	0.39535	0.29545	0.37209
TR66	0.38298	0.45283	0.30952	0.34091	0.36364	0.37209	0.26316	0.36364	0.18919
TR67	0.18605	0.32000	0.21951	0.12500	0.15000	0.15385	0.16216	0.19512	0.18421
TR68	0.25000	0.37255	0.28571	0.19512	0.21951	0.22500	0.23684	0.26190	0.21053
TR69	0.30435	0.41509	0.30233	0.25581	0.27907	0.28571	0.25641	0.27907	0.23077
TR70	0.26667	0.38462	0.30233	0.21429	0.23810	0.24390	0.25641	0.27907	0.23077
TR71	0.26667	0.38462	0.26190	0.21429	0.23810	0.24390	0.21053	0.23810	0.18421
TR72	0.25581	0.38000	0.20513	0.20000	0.22500	0.23077	0.08824	0.26829	0.11429
TR73	0.28889	0.40385	0.24390	0.23810	0.26190	0.26829	0.13889	0.26190	0.16216
TR74	0.24444	0.36538	0.23810	0.19048	0.21429	0.21951	0.18421	0.21429	0.20513
TR75	0.31111	0.42308	0.22500	0.26190	0.28571	0.29268	0.16667	0.28571	0.13889
TR76	0.27907	0.40000	0.23077	0.22500	0.25000	0.25641	0.11765	0.29268	0.08824
TR77	0.35556	0.46154	0.27500	0.30952	0.33333	0.34146	0.22222	0.33333	0.14286
TR78	0.34783	0.45283	0.26829	0.30233	0.32558	0.33333	0.21622	0.36364	0.13889
TR79	0.46000	0.54386	0.40000	0.39130	0.41304	0.42222	0.32500	0.41304	0.30000
TR80	0.30435	0.41509	0.34091	0.21429	0.23810	0.24390	0.25641	0.23810	0.27500
TR81	0.31818	0.43137	0.23077	0.26829	0.29268	0.30000	0.17143	0.33333	0.14286
TR82	0.34884	0.46000	0.30769	0.30000	0.32500	0.33333	0.20588	0.36585	0.17647
TW116	0.36735	0.43636	0.40426	0.36170	0.38298	0.42553	0.44444	0.41667	0.48936
TW117	0.18605	0.32000	0.17500	0.12500	0.15000	0.15385	0.11111	0.19512	0.13514
TW118	0.42222	0.51923	0.39024	0.34146	0.36585	0.37500	0.25714	0.36585	0.32432
TW119	0.36364	0.47059	0.32500	0.31707	0.34146	0.35000	0.22857	0.38095	0.25000
TW120	0.37778	0.48077	0.34146	0.33333	0.35714	0.36585	0.25000	0.39535	0.27027

JACCARD GENETIC DISTANCES

ENTRY	TR10	TR11	TR12	TR13	TR14	TR15	TR16	TR17	TR18
TR1	.	.	.	.	.	.	.	.	.
TR2	.	.	.	.	.	.	.	.	.
TR3	.	.	.	.	.	.	.	.	.
TR4	.	.	.	.	.	.	.	.	.
TR5	.	.	.	.	.	.	.	.	.
TR6	.	.	.	.	.	.	.	.	.
TR7	.	.	.	.	.	.	.	.	.
TR8	.	.	.	.	.	.	.	.	.
TR9	.	.	.	.	.	.	.	.	.
TR10	0.00000	.	.	.	.	.	.	.	.
TR11	0.20513	0.00000	.	.	.	.	.	.	.
TR12	0.19444	0.13889	0.00000	.	.	.	.	.	.
TR13	0.35000	0.21053	0.34211	0.00000	.	.	.	.	.
TR14	0.12821	0.21429	0.25000	0.30952	0.00000	.	.	.	.
TR15	0.32500	0.18421	0.31579	0.03030	0.28571	0.00000	.	.	.
TR16	0.29268	0.20000	0.32500	0.25641	0.29545	0.23077	0.00000	.	.
TR17	0.31707	0.17949	0.30769	0.13889	0.27907	0.11111	0.13158	0.00000	.
TR18	0.38462	0.28947	0.42105	0.09375	0.38095	0.12121	0.24324	0.17143	0.00000
TR20	0.22500	0.07895	0.21053	0.18421	0.19048	0.15789	0.12821	0.10526	0.21622
TR21	0.35000	0.21053	0.34211	0.06061	0.30952	0.08824	0.21053	0.08571	0.09375
TR22	0.41860	0.29268	0.41463	0.16667	0.37778	0.18919	0.33333	0.23077	0.25000
TR23	0.40476	0.27500	0.40000	0.14286	0.36364	0.16667	0.23077	0.16216	0.22857
TR24	0.31707	0.13158	0.26316	0.23684	0.27907	0.21053	0.17949	0.20513	0.31579
TR25	0.46512	0.34146	0.46341	0.22222	0.42222	0.24324	0.34146	0.28205	0.30556
TR26	0.44186	0.27500	0.40000	0.28947	0.40000	0.26316	0.35714	0.30000	0.36842
TR27	0.30233	0.17073	0.29268	0.22500	0.26667	0.20000	0.21429	0.19512	0.30000
TR28	0.41860	0.29268	0.41463	0.35000	0.41304	0.32500	0.29268	0.31707	0.38462
TR29	0.48837	0.36585	0.48780	0.25000	0.44444	0.27027	0.36585	0.30769	0.33333
TR30	0.38095	0.25000	0.37500	0.30769	0.34091	0.28205	0.25000	0.27500	0.38462
TR31	0.40909	0.28571	0.40476	0.21053	0.36957	0.18421	0.28571	0.17949	0.28947
TR32	0.50000	0.38095	0.50000	0.27027	0.45652	0.28947	0.38095	0.32500	0.35135
TR33	0.42222	0.26190	0.38095	0.35714	0.38298	0.33333	0.26190	0.28571	0.42857
TR34	0.34091	0.21429	0.33333	0.26829	0.30435	0.24390	0.21429	0.23810	0.34146
TR35	0.39535	0.26829	0.39024	0.28205	0.35556	0.25641	0.22500	0.20513	0.35897
TR36	0.42222	0.26190	0.38095	0.27500	0.38298	0.25000	0.21951	0.24390	0.30769
TR37	0.46667	0.30952	0.42857	0.23684	0.42553	0.25641	0.34884	0.25000	0.31579
TR38	0.44186	0.31707	0.43902	0.28947	0.40000	0.26316	0.31707	0.25641	0.36842
TR41	0.37209	0.20000	0.32500	0.30000	0.33333	0.27500	0.24390	0.26829	0.37500
TR39	0.50000	0.38636	0.50000	0.28205	0.45833	0.30000	0.34884	0.29268	0.35897
TR40	0.30952	0.17500	0.30000	0.23077	0.27273	0.20513	0.21951	0.20000	0.30769
TR42	0.42857	0.30000	0.38462	0.31579	0.42222	0.28947	0.30000	0.28205	0.39474
TR43	0.41304	0.29545	0.40909	0.34884	0.37500	0.32558	0.33333	0.31818	0.41860
TR44	0.39024	0.21053	0.34211	0.17143	0.34884	0.14286	0.25641	0.18919	0.25714

JACCARD GENETIC DISTANCES

ENTRY	TR10	TR11	TR12	TR13	TR14	TR15	TR16	TR17	TR18
TR45	0.43182	0.30952	0.42857	0.18919	0.39130	0.21053	0.26829	0.20513	0.27027
TR46	0.25000	0.15385	0.23684	0.25641	0.25581	0.23077	0.15385	0.22500	0.28947
TR47	0.36585	0.27500	0.40000	0.14286	0.36364	0.16667	0.23077	0.21053	0.17647
TR48	0.39535	0.26829	0.39024	0.18919	0.35556	0.21053	0.26829	0.25000	0.27027
TR49	0.21951	0.17073	0.29268	0.26829	0.22727	0.24390	0.12500	0.19512	0.30000
TR50	0.50000	0.34884	0.46512	0.28205	0.45833	0.30000	0.38636	0.33333	0.35897
TR51	0.51111	0.35714	0.47619	0.28947	0.46809	0.30769	0.35714	0.30000	0.36842
TR52	0.45238	0.36585	0.48780	0.25000	0.44444	0.27027	0.32500	0.30769	0.28571
TR53	0.45455	0.29268	0.37500	0.35000	0.41304	0.32500	0.29268	0.27500	0.42500
TR54	0.35714	0.17949	0.30769	0.23684	0.31818	0.21053	0.22500	0.25000	0.31579
TR55	0.38095	0.25000	0.37500	0.16667	0.34091	0.13889	0.20513	0.13514	0.25000
TR56	0.36585	0.27500	0.40000	0.19444	0.36364	0.21622	0.18421	0.16216	0.22857
TR57	0.41860	0.33333	0.45238	0.26316	0.41304	0.23684	0.25000	0.23077	0.29730
TR58	0.39024	0.30000	0.38462	0.22222	0.38636	0.19444	0.25641	0.23684	0.25714
TR59	0.33333	0.28571	0.36585	0.34146	0.33333	0.31707	0.24390	0.30952	0.37500
TR60	0.35714	0.26829	0.35000	0.28205	0.35556	0.25641	0.22500	0.29268	0.31579
TR61	0.37209	0.32558	0.40476	0.41860	0.33333	0.39535	0.32558	0.38636	0.45238
TR62	0.30952	0.26190	0.34146	0.31707	0.31111	0.29268	0.21951	0.28571	0.35000
TR63	0.50000	0.37500	0.46154	0.25714	0.48889	0.27778	0.37500	0.35897	0.29412
TR64	0.33333	0.24390	0.32500	0.30000	0.33333	0.27500	0.20000	0.26829	0.33333
TR65	0.34884	0.30233	0.38095	0.39535	0.31111	0.37209	0.30233	0.36364	0.42857
TR66	0.38095	0.33333	0.45238	0.21622	0.37778	0.23684	0.15789	0.18421	0.20000
TR67	0.25000	0.10526	0.23684	0.16216	0.21429	0.13514	0.10526	0.08108	0.19444
TR68	0.27500	0.17949	0.30769	0.23684	0.27907	0.21053	0.08108	0.15789	0.22222
TR69	0.33333	0.24390	0.36585	0.25641	0.33333	0.23077	0.05405	0.13158	0.24324
TR70	0.29268	0.20000	0.32500	0.25641	0.29545	0.23077	0.00000	0.13158	0.24324
TR71	0.29268	0.20000	0.32500	0.21053	0.29545	0.18421	0.10526	0.08108	0.19444
TR72	0.32500	0.18421	0.31579	0.08824	0.28571	0.05882	0.18421	0.05714	0.12121
TR73	0.35714	0.22500	0.35000	0.13889	0.31818	0.11111	0.26829	0.15789	0.22222
TR74	0.30952	0.17500	0.30000	0.18421	0.27273	0.15789	0.12821	0.15385	0.26316
TR75	0.38095	0.25000	0.37500	0.11429	0.34091	0.13889	0.20513	0.13514	0.14706
TR76	0.30769	0.21053	0.34211	0.11765	0.30952	0.08824	0.16216	0.13889	0.15152
TR77	0.39024	0.30000	0.42500	0.17143	0.38636	0.19444	0.21053	0.18919	0.20588
TR78	0.41860	0.29268	0.41463	0.16667	0.37778	0.18919	0.25000	0.18421	0.20000
TR79	0.53191	0.38636	0.50000	0.28205	0.48980	0.30000	0.38636	0.33333	0.35897
TR80	0.37209	0.20000	0.32500	0.30000	0.33333	0.27500	0.24390	0.26829	0.37500
TR81	0.39024	0.25641	0.38462	0.11765	0.34884	0.14286	0.25641	0.18919	0.20588
TR82	0.38462	0.28947	0.42105	0.20588	0.38095	0.17647	0.24324	0.17143	0.18750
TW116	0.53061	0.39130	0.50000	0.47826	0.46000	0.45652	0.42553	0.44681	0.54348
TW117	0.25000	0.10526	0.23684	0.11111	0.21429	0.08333	0.15385	0.08108	0.19444
TW118	0.50000	0.33333	0.46154	0.30556	0.45455	0.27778	0.37500	0.31579	0.38889
TW119	0.43902	0.30769	0.43590	0.22857	0.39535	0.20000	0.26316	0.19444	0.26471
TW120	0.45238	0.32500	0.45000	0.25000	0.40909	0.22222	0.28205	0.21622	0.28571

Jaccard Genetic Distances

ENTRY	TR20	TR21	TR22	TR23	TR24	TR25	TR26	TR27	TR28
TR1	.	.	.	.	.	.	.	.	.
TR2	.	.	.	.	.	.	.	.	.
TR3	.	.	.	.	.	.	.	.	.
TR4	.	.	.	.	.	.	.	.	.
TR5	.	.	.	.	.	.	.	.	.
TR6	.	.	.	.	.	.	.	.	.
TR7	.	.	.	.	.	.	.	.	.
TR8	.	.	.	.	.	.	.	.	.
TR9	.	.	.	.	.	.	.	.	.
TR10	.	.	.	.	.	.	.	.	.
TR11	.	.	.	.	.	.	.	.	.
TR12	.	.	.	.	.	.	.	.	.
TR13	.	.	.	.	.	.	.	.	.
TR14	.	.	.	.	.	.	.	.	.
TR15	.	.	.	.	.	.	.	.	.
TR16	.	.	.	.	.	.	.	.	.
TR17	.	.	.	.	.	.	.	.	.
TR18	.	.	.	.	.	.	.	.	.
TR20	0.00000	.	.	.	.	.	.	.	.
TR21	0.13514	0.00000	.	.	.	.	.	.	.
TR22	0.26829	0.16667	0.00000	.	.	.	.	.	.
TR23	0.25000	0.14286	0.18919	0.00000	.	.	.	.	.
TR24	0.15385	0.23684	0.23077	0.21053	0.00000	.	.	.	.
TR25	0.31707	0.22222	0.16667	0.19444	0.23684	0.00000	.	.	.
TR26	0.29268	0.28947	0.23684	0.30769	0.21053	0.14286	0.00000	.	.
TR27	0.14634	0.22500	0.21951	0.20000	0.10256	0.22500	0.20000	0.00000	.
TR28	0.30952	0.35000	0.25641	0.28205	0.18421	0.26316	0.23684	0.17500	0.00000
TR29	0.34146	0.25000	0.19444	0.22222	0.26316	0.09091	0.17143	0.25000	0.28947
TR30	0.22500	0.30769	0.25641	0.28205	0.13514	0.16667	0.13889	0.12821	0.21053
TR31	0.26190	0.21053	0.20513	0.18421	0.26829	0.30000	0.31707	0.25581	0.33333
TR32	0.35714	0.27027	0.21622	0.24324	0.28205	0.17143	0.24324	0.30952	0.35000
TR33	0.27907	0.35714	0.26829	0.25000	0.15385	0.23077	0.20513	0.14634	0.13158
TR34	0.19048	0.26829	0.26190	0.20000	0.10256	0.22500	0.24390	0.05000	0.17500
TR35	0.24390	0.28205	0.27500	0.16216	0.20513	0.23684	0.25641	0.15000	0.18421
TR36	0.23810	0.23077	0.34884	0.25000	0.24390	0.31707	0.29268	0.23256	0.26829
TR37	0.32558	0.23684	0.18421	0.21053	0.29268	0.28205	0.30000	0.27907	0.23077
TR38	0.29268	0.28947	0.28205	0.26316	0.30000	0.19444	0.16667	0.24390	0.28205
TR41	0.21951	0.30000	0.29268	0.31707	0.17949	0.34146	0.31707	0.25581	0.25000
TR39	0.36364	0.28205	0.27500	0.21053	0.33333	0.18919	0.30000	0.27907	0.27500
TR40	0.15000	0.23077	0.26829	0.29268	0.20000	0.31707	0.29268	0.19048	0.30952
TR42	0.31707	0.31579	0.30769	0.28947	0.23684	0.22222	0.19444	0.26829	0.21622
TR43	0.27273	0.34884	0.30233	0.36364	0.23810	0.26829	0.20000	0.18605	0.30233
TR44	0.23077	0.17143	0.26316	0.19444	0.18919	0.27027	0.28947	0.26829	0.30769

JACCARD GENETIC DISTANCES

ENTRY	TR20	TR21	TR22	TR23	TR24	TR25	TR26	TR27	TR28
TR45	0.28571	0.18919	0.23077	0.16216	0.29268	0.28205	0.38095	0.31818	0.35714
TR46	0.12821	0.25641	0.29268	0.27500	0.17949	0.34146	0.35714	0.21429	0.25000
TR47	0.25000	0.14286	0.18919	0.16667	0.25641	0.24324	0.35000	0.28571	0.28205
TR48	0.24390	0.18919	0.23077	0.21053	0.25000	0.23684	0.30000	0.23810	0.27500
TR49	0.14634	0.26829	0.30233	0.24390	0.19512	0.34884	0.36364	0.22727	0.26190
TR50	0.36364	0.28205	0.27500	0.30000	0.33333	0.32500	0.38095	0.39130	0.35714
TR51	0.37209	0.28947	0.28205	0.16667	0.30000	0.28947	0.35000	0.32558	0.28205
TR52	0.34146	0.25000	0.19444	0.22222	0.26316	0.14706	0.22222	0.29268	0.28947
TR53	0.30952	0.35000	0.34146	0.23684	0.23077	0.35000	0.36585	0.26190	0.25641
TR54	0.20000	0.23684	0.31707	0.25641	0.15789	0.28205	0.25641	0.19512	0.27500
TR55	0.22500	0.16667	0.16216	0.08571	0.18421	0.21622	0.28205	0.21951	0.25641
TR56	0.25000	0.19444	0.23684	0.16667	0.25641	0.24324	0.35000	0.28571	0.28205
TR57	0.30952	0.26316	0.21053	0.23684	0.31707	0.30769	0.36585	0.34091	0.30000
TR58	0.27500	0.22222	0.16667	0.24324	0.28205	0.27027	0.33333	0.30952	0.26316
TR59	0.26190	0.34146	0.33333	0.35714	0.26829	0.41860	0.43182	0.29545	0.33333
TR60	0.24390	0.28205	0.27500	0.30000	0.25000	0.36585	0.38095	0.27907	0.31707
TR61	0.30233	0.41860	0.37209	0.43182	0.34884	0.41860	0.43182	0.36957	0.37209
TR62	0.23810	0.31707	0.30952	0.33333	0.24390	0.39535	0.40909	0.27273	0.30952
TR63	0.39024	0.30556	0.25000	0.32432	0.35897	0.35135	0.41026	0.41860	0.34211
TR64	0.21951	0.30000	0.29268	0.31707	0.22500	0.38095	0.39535	0.25581	0.29268
TR65	0.27907	0.39535	0.30952	0.37209	0.28571	0.39535	0.40909	0.31111	0.30952
TR66	0.26829	0.16667	0.30000	0.18919	0.31707	0.30769	0.40476	0.34091	0.38095
TR67	0.02703	0.11111	0.25000	0.23077	0.13158	0.30000	0.27500	0.12500	0.29268
TR68	0.10526	0.18919	0.31707	0.30000	0.20513	0.36585	0.34146	0.19512	0.31707
TR69	0.17500	0.21053	0.29268	0.23077	0.22500	0.34146	0.35714	0.25581	0.25000
TR70	0.12821	0.21053	0.33333	0.23077	0.17949	0.34146	0.35714	0.21429	0.29268
TR71	0.12821	0.16216	0.25000	0.23077	0.22500	0.34146	0.35714	0.21429	0.29268
TR72	0.10811	0.03030	0.18919	0.16667	0.21053	0.24324	0.26316	0.20000	0.32500
TR73	0.20000	0.13889	0.18421	0.21053	0.25000	0.23684	0.25641	0.19512	0.27500
TR74	0.15000	0.18421	0.22500	0.20513	0.15385	0.27500	0.29268	0.19048	0.22500
TR75	0.17949	0.05882	0.16216	0.18919	0.27500	0.26316	0.32500	0.26190	0.34146
TR76	0.18421	0.11765	0.21622	0.14286	0.18919	0.22222	0.28947	0.22500	0.26316
TR77	0.27500	0.17143	0.21622	0.14286	0.28205	0.22222	0.33333	0.30952	0.30769
TR78	0.22500	0.11429	0.21053	0.18919	0.23077	0.21622	0.32500	0.26190	0.34146
TR79	0.40000	0.32500	0.27500	0.25641	0.33333	0.28205	0.34146	0.31818	0.23077
TR80	0.21951	0.30000	0.25000	0.27500	0.08108	0.30000	0.27500	0.17073	0.15789
TR81	0.23077	0.11765	0.16667	0.08824	0.23684	0.17143	0.28947	0.22500	0.26316
TR82	0.21622	0.15152	0.25000	0.22857	0.27027	0.30556	0.32432	0.25641	0.34211
TW116	0.40426	0.47826	0.43478	0.45652	0.34091	0.47826	0.45652	0.39583	0.40000
TW117	0.07895	0.11111	0.20513	0.18421	0.13158	0.25641	0.27500	0.12500	0.29268
TW118	0.35000	0.30556	0.29730	0.32432	0.31579	0.30556	0.32432	0.38095	0.34211
TW119	0.23684	0.17647	0.31579	0.20000	0.28947	0.27778	0.34211	0.27500	0.35897
TW120	0.25641	0.20000	0.33333	0.22222	0.30769	0.29730	0.35897	0.29268	0.37500

JACCARD GENETIC DISTANCES

ENTRY	TR29	TR30	TR31	TR32	TR33	TR34	TR35	TR36	TR37
TR1	.	.	.	.	.	.	.	.	.
TR2	.	.	.	.	.	.	.	.	.
TR3	.	.	.	.	.	.	.	.	.
TR4	.	.	.	.	.	.	.	.	.
TR5	.	.	.	.	.	.	.	.	.
TR6	.	.	.	.	.	.	.	.	.
TR7	.	.	.	.	.	.	.	.	.
TR8	.	.	.	.	.	.	.	.	.
TR9	.	.	.	.	.	.	.	.	.
TR10	.	.	.	.	.	.	.	.	.
TR11	.	.	.	.	.	.	.	.	.
TR12	.	.	.	.	.	.	.	.	.
TR13	.	.	.	.	.	.	.	.	.
TR14	.	.	.	.	.	.	.	.	.
TR15	.	.	.	.	.	.	.	.	.
TR16	.	.	.	.	.	.	.	.	.
TR17	.	.	.	.	.	.	.	.	.
TR18	.	.	.	.	.	.	.	.	.
TR20	.	.	.	.	.	.	.	.	.
TR21	.	.	.	.	.	.	.	.	.
TR22	.	.	.	.	.	.	.	.	.
TR23	.	.	.	.	.	.	.	.	.
TR24	.	.	.	.	.	.	.	.	.
TR25	.	.	.	.	.	.	.	.	.
TR26	.	.	.	.	.	.	.	.	.
TR27	.	.	.	.	.	.	.	.	.
TR28	.	.	.	.	.	.	.	.	.
TR29	0.00000	.	.	.	.	.	.	.	.
TR30	0.14286	0.00000	.	.	.	.	.	.	.
TR31	0.32500	0.33333	0.00000	.	.	.	.	.	.
TR32	0.14706	0.21622	0.34146	0.00000	.	.	.	.	.
TR33	0.25641	0.13158	0.30233	0.31707	0.00000	.	.	.	.
TR34	0.20513	0.12821	0.29545	0.30952	0.14634	0.00000	.	.	.
TR35	0.26316	0.18421	0.26829	0.32500	0.15385	0.15000	0.00000	.	.
TR36	0.34146	0.30952	0.30233	0.39535	0.27907	0.23256	0.24390	0.00000	.
TR37	0.26316	0.35714	0.26829	0.32500	0.24390	0.27907	0.29268	0.32558	0.00000
TR38	0.27027	0.23684	0.27500	0.33333	0.25000	0.28571	0.21053	0.33333	0.30000
TR41	0.36585	0.25000	0.24390	0.38095	0.21951	0.25581	0.26829	0.30233	0.30952
TR39	0.16667	0.27500	0.30952	0.28205	0.24390	0.23810	0.25000	0.28571	0.15789
TR40	0.34146	0.22500	0.26190	0.35714	0.23810	0.23256	0.28571	0.31818	0.32558
TR42	0.25000	0.21622	0.34146	0.31579	0.18421	0.26829	0.23684	0.27500	0.23684
TR43	0.20513	0.12821	0.33333	0.30952	0.23256	0.18605	0.27907	0.34783	0.35556
TR44	0.29730	0.30769	0.16216	0.31579	0.31707	0.26829	0.28205	0.23077	0.28205

Jaccard Genetic Distances

ENTRY	TR29	TR30	TR31	TR32	TR33	TR34	TR35	TR36	TR37
TR45	0.30769	0.35714	0.13158	0.32500	0.32558	0.31818	0.29268	0.32558	0.25000
TR46	0.36585	0.25000	0.28571	0.38095	0.26190	0.21429	0.26829	0.30233	0.34884
TR47	0.27027	0.32500	0.23077	0.28947	0.33333	0.28571	0.30000	0.29268	0.25641
TR48	0.26316	0.27500	0.17949	0.32500	0.28571	0.23810	0.25000	0.24390	0.29268
TR49	0.37209	0.26190	0.25581	0.38636	0.23256	0.22727	0.23810	0.31111	0.31818
TR50	0.35000	0.39535	0.34884	0.36585	0.36364	0.39130	0.37209	0.36364	0.29268
TR51	0.27027	0.36585	0.27500	0.33333	0.29268	0.28571	0.25641	0.29268	0.16216
TR52	0.17647	0.24324	0.32500	0.20000	0.34146	0.29268	0.30769	0.38095	0.30769
TR53	0.33333	0.30000	0.29268	0.39024	0.22500	0.21951	0.23077	0.30952	0.23077
TR54	0.30769	0.23077	0.22500	0.36585	0.24390	0.19512	0.25000	0.20000	0.33333
TR55	0.24324	0.25641	0.10811	0.26316	0.22500	0.21951	0.18421	0.26829	0.23077
TR56	0.27027	0.28205	0.23077	0.28947	0.25000	0.28571	0.25641	0.33333	0.25641
TR57	0.33333	0.34146	0.15789	0.35000	0.30952	0.34091	0.27500	0.34884	0.27500
TR58	0.29730	0.30769	0.25641	0.31579	0.31707	0.30952	0.28205	0.31707	0.28205
TR59	0.44186	0.33333	0.28571	0.45455	0.34091	0.29545	0.34884	0.37778	0.42222
TR60	0.39024	0.31707	0.22500	0.40476	0.32558	0.27907	0.33333	0.32558	0.37209
TR61	0.44186	0.33333	0.36364	0.45455	0.34091	0.36957	0.38636	0.44681	0.45652
TR62	0.41860	0.30952	0.26190	0.43182	0.31818	0.27273	0.32558	0.35556	0.40000
TR63	0.37838	0.42500	0.28947	0.39474	0.39024	0.41860	0.40000	0.39024	0.31579
TR64	0.40476	0.29268	0.24390	0.41860	0.30233	0.25581	0.30952	0.34091	0.38636
TR65	0.41860	0.30952	0.30233	0.43182	0.31818	0.31111	0.32558	0.42553	0.43478
TR66	0.33333	0.38095	0.29268	0.35000	0.38636	0.34091	0.31707	0.26829	0.31707
TR67	0.32500	0.20513	0.24390	0.34146	0.26190	0.17073	0.22500	0.21951	0.30952
TR68	0.39024	0.27500	0.30952	0.40476	0.32558	0.23810	0.29268	0.24390	0.37209
TR69	0.36585	0.29268	0.24390	0.38095	0.26190	0.25581	0.22500	0.21951	0.30952
TR70	0.36585	0.25000	0.28571	0.38095	0.26190	0.21429	0.22500	0.21951	0.34884
TR71	0.36585	0.29268	0.20000	0.38095	0.26190	0.25581	0.26829	0.30233	0.26829
TR72	0.27027	0.28205	0.18421	0.28947	0.33333	0.24390	0.25641	0.20513	0.25641
TR73	0.26316	0.27500	0.17949	0.32500	0.28571	0.23810	0.25000	0.24390	0.25000
TR74	0.30000	0.22500	0.21951	0.31707	0.23810	0.19048	0.20000	0.19512	0.28571
TR75	0.28947	0.34146	0.20513	0.30769	0.34884	0.30233	0.31707	0.22500	0.23077
TR76	0.25000	0.26316	0.21053	0.27027	0.31707	0.22500	0.23684	0.23077	0.28205
TR77	0.25000	0.30769	0.21053	0.27027	0.27500	0.30952	0.28205	0.31707	0.23684
TR78	0.24324	0.30000	0.29268	0.26316	0.34884	0.26190	0.31707	0.22500	0.27500
TR79	0.30769	0.35714	0.34884	0.36585	0.24390	0.31818	0.29268	0.32558	0.15789
TR80	0.32500	0.20513	0.28571	0.34146	0.17500	0.17073	0.26829	0.30233	0.26829
TR81	0.20000	0.26316	0.25641	0.22222	0.27500	0.22500	0.18919	0.23077	0.18919
TR82	0.33333	0.34211	0.24324	0.35135	0.39024	0.30000	0.31579	0.30769	0.35897
TW116	0.50000	0.40000	0.45833	0.51064	0.36957	0.39583	0.41304	0.46939	0.47917
TW117	0.28205	0.20513	0.20000	0.30000	0.26190	0.17073	0.22500	0.26190	0.26829
TW118	0.33333	0.34211	0.24324	0.35135	0.30769	0.38095	0.35897	0.35000	0.31579
TW119	0.30556	0.31579	0.30769	0.32432	0.32500	0.27500	0.28947	0.23684	0.33333
TW120	0.32432	0.33333	0.32500	0.34211	0.34146	0.29268	0.30769	0.25641	0.35000

JACCARD GENETIC DISTANCES

ENTRY	TR38	TR41	TR39	TR40	TR42	TR43	TR44	TR45	TR46
TR1	.	.	.	.	.	.	.	.	.
TR2	.	.	.	.	.	.	.	.	.
TR3	.	.	.	.	.	.	.	.	.
TR4	.	.	.	.	.	.	.	.	.
TR5	.	.	.	.	.	.	.	.	.
TR6	.	.	.	.	.	.	.	.	.
TR7	.	.	.	.	.	.	.	.	.
TR8	.	.	.	.	.	.	.	.	.
TR9	.	.	.	.	.	.	.	.	.
TR10	.	.	.	.	.	.	.	.	.
TR11	.	.	.	.	.	.	.	.	.
TR12	.	.	.	.	.	.	.	.	.
TR13	.	.	.	.	.	.	.	.	.
TR14	.	.	.	.	.	.	.	.	.
TR15	.	.	.	.	.	.	.	.	.
TR16	.	.	.	.	.	.	.	.	.
TR17	.	.	.	.	.	.	.	.	.
TR18	.	.	.	.	.	.	.	.	.
TR20	.	.	.	.	.	.	.	.	.
TR21	.	.	.	.	.	.	.	.	.
TR22	.	.	.	.	.	.	.	.	.
TR23	.	.	.	.	.	.	.	.	.
TR24	.	.	.	.	.	.	.	.	.
TR25	.	.	.	.	.	.	.	.	.
TR26	.	.	.	.	.	.	.	.	.
TR27	.	.	.	.	.	.	.	.	.
TR28	.	.	.	.	.	.	.	.	.
TR29	.	.	.	.	.	.	.	.	.
TR30	.	.	.	.	.	.	.	.	.
TR31	.	.	.	.	.	.	.	.	.
TR32	.	.	.	.	.	.	.	.	.
TR33	.	.	.	.	.	.	.	.	.
TR34	.	.	.	.	.	.	.	.	.
TR35	.	.	.	.	.	.	.	.	.
TR36	.	.	.	.	.	.	.	.	.
TR37	.	.	.	.	.	.	.	.	.
TR38	0.00000	.	.	.	.	.	.	.	.
TR41	0.35714	0.00000	.	.	.	.	.	.	.
TR39	0.25641	0.38636	0.00000	.	.	.	.	.	.
TR40	0.29268	0.21951	0.36364	0.00000	.	.	.	.	.
TR42	0.19444	0.30000	0.18919	0.31707	0.00000	.	.	.	.
TR43	0.28571	0.33333	0.27907	0.27273	0.30952	0.00000	.	.	.
TR44	0.33333	0.16216	0.32500	0.27500	0.27027	0.38636	0.00000	.	.

JACCARD GENETIC DISTANCES

ENTRY	TR38	TR41	TR39	TR40	TR42	TR43	TR44	TR45	TR46
TR45	0.34146	0.22500	0.29268	0.28571	0.32500	0.39130	0.13889	0.00000	.
TR46	0.35714	0.20000	0.38636	0.17500	0.34146	0.33333	0.25641	0.26829	0.00000
TR47	0.35000	0.27500	0.30000	0.25000	0.33333	0.36364	0.19444	0.11111	0.18421
TR48	0.30000	0.17949	0.29268	0.24390	0.28205	0.35556	0.13889	0.10811	0.22500
TR49	0.32558	0.21429	0.35556	0.19048	0.30952	0.34043	0.26829	0.23810	0.07692
TR50	0.41860	0.30952	0.37209	0.36364	0.36585	0.45833	0.28205	0.29268	0.34884
TR51	0.30769	0.35714	0.16216	0.40909	0.28947	0.36364	0.28947	0.30000	0.39535
TR52	0.31579	0.36585	0.30769	0.38095	0.34211	0.33333	0.29730	0.30769	0.32500
TR53	0.32500	0.29268	0.23077	0.34884	0.30769	0.30233	0.30769	0.35714	0.29268
TR54	0.30000	0.13158	0.33333	0.24390	0.23684	0.31818	0.08571	0.20513	0.22500
TR55	0.23684	0.25000	0.27500	0.22500	0.26316	0.34091	0.16667	0.13514	0.20513
TR56	0.30769	0.27500	0.25641	0.20513	0.28947	0.36364	0.24324	0.16216	0.18421
TR57	0.32500	0.20513	0.31707	0.30952	0.30769	0.41304	0.16667	0.13514	0.25000
TR58	0.33333	0.25641	0.32500	0.27500	0.31579	0.38636	0.22222	0.23684	0.16216
TR59	0.43182	0.20000	0.45652	0.26190	0.41860	0.40426	0.25641	0.26829	0.15385
TR60	0.38095	0.17949	0.40909	0.24390	0.36585	0.39130	0.18919	0.20513	0.13158
TR61	0.43182	0.24390	0.45652	0.30233	0.41860	0.40426	0.34146	0.34884	0.20000
TR62	0.40909	0.17500	0.43478	0.23810	0.39535	0.38298	0.23077	0.24390	0.12821
TR63	0.45000	0.24324	0.40000	0.39024	0.39474	0.48889	0.20588	0.22222	0.28947
TR64	0.39535	0.15385	0.42222	0.21951	0.38095	0.36957	0.21053	0.22500	0.10526
TR65	0.40909	0.21951	0.46809	0.31818	0.43182	0.38298	0.31707	0.32558	0.17500
TR66	0.36585	0.37209	0.31707	0.34884	0.35000	0.44681	0.26316	0.23077	0.29268
TR67	0.27500	0.20000	0.34884	0.12821	0.30000	0.25581	0.21053	0.26829	0.15385
TR68	0.34146	0.26829	0.40909	0.20000	0.36585	0.31818	0.28205	0.33333	0.17949
TR69	0.31707	0.24390	0.34884	0.21951	0.30000	0.36957	0.25641	0.22500	0.15385
TR70	0.31707	0.24390	0.34884	0.21951	0.30000	0.33333	0.25641	0.26829	0.15385
TR71	0.31707	0.24390	0.34884	0.17500	0.34146	0.33333	0.25641	0.22500	0.15385
TR72	0.26316	0.27500	0.30000	0.20513	0.28947	0.32558	0.14286	0.21053	0.23077
TR73	0.25641	0.26829	0.29268	0.20000	0.28205	0.31818	0.18919	0.20513	0.22500
TR74	0.29268	0.17500	0.32558	0.15000	0.27500	0.31111	0.18421	0.20000	0.12821
TR75	0.32500	0.29268	0.31707	0.22500	0.35000	0.37778	0.21622	0.18421	0.25000
TR76	0.28947	0.25641	0.28205	0.23077	0.27027	0.34884	0.11765	0.18919	0.16216
TR77	0.28947	0.30000	0.23684	0.23077	0.27027	0.38636	0.22222	0.13889	0.21053
TR78	0.36585	0.33333	0.27500	0.30952	0.30769	0.34091	0.21622	0.23077	0.29268
TR79	0.34146	0.34884	0.20513	0.40000	0.23684	0.39130	0.32500	0.25000	0.38636
TR80	0.31707	0.20000	0.34884	0.21951	0.25641	0.25581	0.25641	0.26829	0.20000
TR81	0.28947	0.25641	0.18919	0.27500	0.27027	0.34884	0.17143	0.18919	0.25641
TR82	0.32432	0.37500	0.40000	0.30769	0.39474	0.38095	0.25714	0.31579	0.28947
TW116	0.48936	0.31818	0.54000	0.40426	0.47826	0.46000	0.44444	0.47917	0.39130
TW117	0.27500	0.20000	0.30952	0.12821	0.30000	0.25581	0.16216	0.22500	0.15385
TW118	0.36842	0.19444	0.35897	0.30769	0.30556	0.41860	0.15152	0.22222	0.33333
TW119	0.34211	0.35000	0.28947	0.28205	0.32432	0.39535	0.22857	0.28947	0.30769
TW120	0.35897	0.36585	0.30769	0.30000	0.34211	0.40909	0.25000	0.30769	0.32500

JACCARD GENETIC DISTANCES

ENTRY	TR47	TR48	TR49	TR50	TR51	TR52	TR53	TR54	TR55
TR45	.	.	.	.	.	.	.	.	.
TR46	.	.	.	.	.	.	.	.	.
TR47	0.00000	.	.	.	.	.	.	.	.
TR48	0.16216	0.00000	.	.	.	.	.	.	.
TR49	0.20000	0.23810	0.00000	.	.	.	.	.	.
TR50	0.25641	0.29268	0.35556	0.00000	.	.	.	.	.
TR51	0.30769	0.34146	0.36364	0.30000	0.00000	.	.	.	.
TR52	0.22222	0.30769	0.33333	0.35000	0.31579	0.00000	.	.	.
TR53	0.36585	0.39535	0.30233	0.39535	0.13889	0.37500	0.00000	.	.
TR54	0.25641	0.10811	0.23810	0.33333	0.34146	0.35000	0.31707	0.00000	.
TR55	0.13889	0.18421	0.17500	0.27500	0.23684	0.24324	0.25641	0.23077	0.00000
TR56	0.11429	0.21053	0.15385	0.30000	0.30769	0.27027	0.32500	0.30000	0.13889
TR57	0.18919	0.18421	0.21951	0.31707	0.32500	0.28947	0.34146	0.23077	0.16216
TR58	0.14286	0.23684	0.22500	0.28205	0.33333	0.25000	0.30769	0.28205	0.16667
TR59	0.27500	0.22500	0.17073	0.42222	0.46667	0.40476	0.37209	0.22500	0.29268
TR60	0.21053	0.15789	0.19512	0.37209	0.41860	0.35000	0.35714	0.15789	0.23077
TR61	0.35714	0.30952	0.21429	0.45652	0.50000	0.44186	0.40909	0.30952	0.37209
TR62	0.25000	0.20000	0.19048	0.40000	0.44444	0.38095	0.34884	0.20000	0.26829
TR63	0.22857	0.22222	0.34146	0.31579	0.36842	0.33333	0.38462	0.27027	0.29730
TR64	0.23077	0.17949	0.17073	0.38636	0.43182	0.36585	0.33333	0.17949	0.25000
TR65	0.33333	0.28571	0.19048	0.43478	0.44444	0.38095	0.34884	0.28571	0.30952
TR66	0.18919	0.27500	0.26190	0.35714	0.32500	0.28947	0.38095	0.31707	0.21053
TR67	0.23077	0.22500	0.17073	0.34884	0.35714	0.32500	0.29268	0.17949	0.20513
TR68	0.25641	0.29268	0.19512	0.40909	0.41860	0.35000	0.35714	0.25000	0.27500
TR69	0.18421	0.22500	0.12500	0.34884	0.35714	0.32500	0.33333	0.26829	0.15789
TR70	0.23077	0.26829	0.12500	0.38636	0.35714	0.32500	0.29268	0.22500	0.20513
TR71	0.18421	0.26829	0.12500	0.34884	0.35714	0.32500	0.29268	0.26829	0.15789
TR72	0.16667	0.21053	0.24390	0.30000	0.30769	0.27027	0.32500	0.21053	0.13889
TR73	0.16216	0.15789	0.23810	0.29268	0.34146	0.30769	0.35714	0.20513	0.13514
TR74	0.15789	0.15385	0.14634	0.28571	0.33333	0.30000	0.30952	0.20000	0.13158
TR75	0.13889	0.18421	0.26190	0.27500	0.32500	0.28947	0.38095	0.27500	0.16216
TR76	0.08824	0.18919	0.17949	0.28205	0.28947	0.20000	0.30769	0.18919	0.11429
TR77	0.08824	0.18919	0.17949	0.28205	0.28947	0.25000	0.35000	0.28205	0.11429
TR78	0.18919	0.23077	0.30233	0.31707	0.32500	0.24324	0.38095	0.27500	0.21053
TR79	0.25641	0.29268	0.35556	0.33333	0.25641	0.35000	0.31707	0.33333	0.27500
TR80	0.23077	0.26829	0.21429	0.34884	0.31707	0.32500	0.25000	0.22500	0.20513
TR81	0.14286	0.18919	0.26829	0.23684	0.19444	0.20000	0.26316	0.23684	0.16667
TR82	0.22857	0.31579	0.30000	0.40000	0.36842	0.28571	0.38462	0.31579	0.20000
TW116	0.45652	0.44681	0.39583	0.37778	0.48936	0.50000	0.43478	0.41304	0.40000
TW117	0.18421	0.22500	0.17073	0.30952	0.31707	0.28205	0.25000	0.17949	0.15789
TW118	0.27778	0.22222	0.34146	0.31579	0.36842	0.37838	0.38462	0.22222	0.25000
TW119	0.25000	0.28947	0.31707	0.37500	0.34211	0.35135	0.35897	0.28947	0.22222
TW120	0.27027	0.30769	0.33333	0.39024	0.35897	0.36842	0.37500	0.30769	0.24324

JACCARD GENETIC DISTANCES

ENTRY	TR56	TR57	TR58	TR59	TR60	TR61	TR62	TR63	TR64
TR45	.	.	.	.	.	.	.	.	.
TR46	.	.	.	.	.	.	.	.	.
TR47	.	.	.	.	.	.	.	.	.
TR48	.	.	.	.	.	.	.	.	.
TR49	.	.	.	.	.	.	.	.	.
TR50	.	.	.	.	.	.	.	.	.
TR51	.	.	.	.	.	.	.	.	.
TR52	.	.	.	.	.	.	.	.	.
TR53	.	.	.	.	.	.	.	.	.
TR54	.	.	.	.	.	.	.	.	.
TR55	.	.	.	.	.	.	.	.	.
TR56	0.00000	.	.	.	.	.	.	.	.
TR57	0.18919	0.00000	.	.	.	.	.	.	.
TR58	0.19444	0.11429	0.00000	.	.	.	.	.	.
TR59	0.27500	0.20513	0.21053	0.00000	.	.	.	.	.
TR60	0.25641	0.13514	0.13889	0.08108	0.00000	.	.	.	.
TR61	0.31707	0.25000	0.25641	0.15385	0.17949	0.00000	.	.	.
TR62	0.25000	0.17949	0.18421	0.07895	0.05405	0.17500	0.00000	.	.
TR63	0.27778	0.14706	0.15152	0.24324	0.17143	0.28947	0.21622	0.00000	.
TR64	0.23077	0.15789	0.16216	0.05405	0.02778	0.15385	0.02703	0.19444	0.00000
TR65	0.33333	0.22500	0.23077	0.12821	0.15385	0.07895	0.15000	0.26316	0.12821
TR66	0.18919	0.25641	0.26316	0.37209	0.31707	0.44444	0.30952	0.34211	0.33333
TR67	0.23077	0.29268	0.25641	0.24390	0.22500	0.32558	0.21951	0.37500	0.20000
TR68	0.25641	0.31707	0.28205	0.26829	0.25000	0.34884	0.24390	0.40000	0.22500
TR69	0.13514	0.20513	0.21053	0.24390	0.22500	0.32558	0.21951	0.33333	0.20000
TR70	0.18421	0.25000	0.25641	0.24390	0.22500	0.32558	0.21951	0.37500	0.20000
TR71	0.13514	0.20513	0.21053	0.24390	0.22500	0.32558	0.21951	0.33333	0.20000
TR72	0.21622	0.23684	0.19444	0.31707	0.25641	0.39535	0.29268	0.32432	0.27500
TR73	0.21053	0.23077	0.18919	0.30952	0.25000	0.38636	0.28571	0.31579	0.26829
TR74	0.15789	0.22500	0.18421	0.21951	0.20000	0.30233	0.19512	0.30769	0.17500
TR75	0.18919	0.25641	0.21622	0.33333	0.27500	0.40909	0.30952	0.29730	0.29268
TR76	0.14286	0.16667	0.11765	0.25641	0.18919	0.34146	0.23077	0.25714	0.21053
TR77	0.03030	0.16667	0.17143	0.30000	0.23684	0.34146	0.27500	0.25714	0.25641
TR78	0.23684	0.30000	0.26316	0.37209	0.31707	0.44444	0.34884	0.34211	0.33333
TR79	0.30000	0.31707	0.32500	0.45652	0.40909	0.48936	0.43478	0.31579	0.42222
TR80	0.27500	0.33333	0.30000	0.28571	0.26829	0.36364	0.26190	0.37500	0.24390
TR81	0.19444	0.21622	0.17143	0.34146	0.28205	0.38095	0.31707	0.25714	0.30000
TR82	0.27778	0.29730	0.25714	0.37500	0.31579	0.45238	0.35000	0.38889	0.33333
TW116	0.45652	0.46809	0.44444	0.45833	0.44681	0.45833	0.43750	0.51111	0.42553
TW117	0.18421	0.25000	0.21053	0.24390	0.22500	0.32558	0.21951	0.33333	0.20000
TW118	0.27778	0.20000	0.25714	0.28947	0.27027	0.33333	0.30769	0.24242	0.28947
TW119	0.25000	0.31579	0.27778	0.39024	0.33333	0.42857	0.36585	0.40541	0.35000
TW120	0.27027	0.33333	0.29730	0.40476	0.35000	0.44186	0.38095	0.42105	0.36585

JACCARD GENETIC DISTANCES

ENTRY	TR65	TR66	TR67	TR68	TR69	TR70	TR71	TR72	TR73
TR45	.	.	.	.	.	.	.	.	.
TR46	.	.	.	.	.	.	.	.	.
TR47	.	.	.	.	.	.	.	.	.
TR48	.	.	.	.	.	.	.	.	.
TR49	.	.	.	.	.	.	.	.	.
TR50	.	.	.	.	.	.	.	.	.
TR51	.	.	.	.	.	.	.	.	.
TR52	.	.	.	.	.	.	.	.	.
TR53	.	.	.	.	.	.	.	.	.
TR54	.	.	.	.	.	.	.	.	.
TR55	.	.	.	.	.	.	.	.	.
TR56	.	.	.	.	.	.	.	.	.
TR57	.	.	.	.	.	.	.	.	.
TR58	.	.	.	.	.	.	.	.	.
TR59	.	.	.	.	.	.	.	.	.
TR60	.	.	.	.	.	.	.	.	.
TR61	.	.	.	.	.	.	.	.	.
TR62	.	.	.	.	.	.	.	.	.
TR63	.	.	.	.	.	.	.	.	.
TR64	.	.	.	.	.	.	.	.	.
TR65	0.00000	.	.	.	.	.	.	.	.
TR66	0.42222	0.00000	.	.	.	.	.	.	.
TR67	0.30233	0.25000	0.00000	.	.	.	.	.	.
TR68	0.32558	0.23077	0.08108	0.00000	.	.	.	.	.
TR69	0.30233	0.15789	0.15385	0.13158	0.00000	.	.	.	.
TR70	0.30233	0.15789	0.10526	0.08108	0.05405	0.00000	.	.	.
TR71	0.30233	0.20513	0.10526	0.13158	0.10526	0.10526	0.00000	.	.
TR72	0.37209	0.18919	0.08333	0.16216	0.18421	0.18421	0.13514	0.00000	.
TR73	0.36364	0.27500	0.17949	0.25000	0.22500	0.26829	0.17949	0.11111	0.00000
TR74	0.27907	0.22500	0.12821	0.15385	0.07895	0.12821	0.17500	0.15789	0.15385
TR75	0.38636	0.16216	0.15789	0.18421	0.15789	0.20513	0.15789	0.08571	0.13514
TR76	0.31707	0.16667	0.16216	0.18919	0.16216	0.16216	0.16216	0.08824	0.13889
TR77	0.35714	0.16667	0.25641	0.28205	0.16216	0.21053	0.16216	0.19444	0.18919
TR78	0.42222	0.21053	0.20513	0.27500	0.25000	0.25000	0.25000	0.13889	0.23077
TR79	0.46809	0.35714	0.38636	0.44444	0.34884	0.38636	0.34884	0.34146	0.29268
TR80	0.30233	0.37209	0.20000	0.26829	0.24390	0.24390	0.24390	0.27500	0.26829
TR81	0.35714	0.21622	0.21053	0.28205	0.25641	0.25641	0.25641	0.14286	0.18919
TR82	0.39024	0.25000	0.19444	0.17143	0.24324	0.24324	0.19444	0.12121	0.22222
TW116	0.40426	0.53061	0.39130	0.44681	0.42553	0.42553	0.42553	0.45652	0.44681
TW117	0.30233	0.25000	0.05405	0.13158	0.20000	0.15385	0.10526	0.08333	0.13158
TW118	0.35000	0.38462	0.33333	0.40000	0.33333	0.37500	0.33333	0.27778	0.22222
TW119	0.44186	0.27027	0.21622	0.28947	0.26316	0.26316	0.26316	0.14706	0.19444
TW120	0.45455	0.28947	0.23684	0.30769	0.28205	0.28205	0.28205	0.17143	0.21622

JACCARD GENETIC DISTANCES

ENTRY	TR74	TR75	TR76	TR77	TR78	TR79	TR80	TR81	TR82
TR45	.	.	.	.	.	.	.	.	.
TR46	.	.	.	.	.	.	.	.	.
TR47	.	.	.	.	.	.	.	.	.
TR48	.	.	.	.	.	.	.	.	.
TR49	.	.	.	.	.	.	.	.	.
TR50	.	.	.	.	.	.	.	.	.
TR51	.	.	.	.	.	.	.	.	.
TR52	.	.	.	.	.	.	.	.	.
TR53	.	.	.	.	.	.	.	.	.
TR54	.	.	.	.	.	.	.	.	.
TR55	.	.	.	.	.	.	.	.	.
TR56	.	.	.	.	.	.	.	.	.
TR57	.	.	.	.	.	.	.	.	.
TR58	.	.	.	.	.	.	.	.	.
TR59	.	.	.	.	.	.	.	.	.
TR60	.	.	.	.	.	.	.	.	.
TR61	.	.	.	.	.	.	.	.	.
TR62	.	.	.	.	.	.	.	.	.
TR63	.	.	.	.	.	.	.	.	.
TR64	.	.	.	.	.	.	.	.	.
TR65	.	.	.	.	.	.	.	.	.
TR66	.	.	.	.	.	.	.	.	.
TR67	.	.	.	.	.	.	.	.	.
TR68	.	.	.	.	.	.	.	.	.
TR69	.	.	.	.	.	.	.	.	.
TR70	.	.	.	.	.	.	.	.	.
TR71	.	.	.	.	.	.	.	.	.
TR72	.	.	.	.	.	.	.	.	.
TR73	.	.	.	.	.	.	.	.	.
TR74	0.00000	.	.	.	.	.	.	.	.
TR75	0.13158	0.00000	.	.	.	.	.	.	.
TR76	0.13514	0.16667	0.00000	.	.	.	.	.	.
TR77	0.18421	0.16667	0.11765	0.00000	.	.	.	.	.
TR78	0.22500	0.16216	0.16667	0.21622	0.00000	.	.	.	.
TR79	0.32558	0.31707	0.32500	0.28205	0.31707	0.00000	.	.	.
TR80	0.17500	0.29268	0.25641	0.30000	0.29268	0.26829	0.00000	.	.
TR81	0.18421	0.16667	0.11765	0.17143	0.16667	0.23684	0.30000	0.00000	.
TR82	0.26316	0.20000	0.15152	0.25714	0.25000	0.43920	0.33333	0.25714	0.00000
TW116	0.36957	0.46809	0.44444	0.47826	0.50000	0.51020	0.35556	0.44444	0.51111
TW117	0.12821	0.15789	0.11111	0.21053	0.20513	0.34884	0.20000	0.16216	0.19444
TW118	0.26316	0.29730	0.25714	0.25714	0.34211	0.35897	0.33333	0.25714	0.38889
TW119	0.23684	0.22222	0.17647	0.22857	0.22222	0.37500	0.35000	0.17647	0.26471
TW120	0.25641	0.24324	0.20000	0.25000	0.24324	0.39024	0.36585	0.20000	0.28571

JACCARD GENETIC DISTANCES

ENTRY	TW116	TW117	TW118	TW119	TW120
TR45	.	.	.	.	.
TR46	.	.	.	.	.
TR47	.	.	.	.	.
TR48	.	.	.	.	.
TR49	.	.	.	.	.
TR50	.	.	.	.	.
TR51	.	.	.	.	.
TR52	.	.	.	.	.
TR53	.	.	.	.	.
TR54	.	.	.	.	.
TR55	.	.	.	.	.
TR56	.	.	.	.	.
TR57	.	.	.	.	.
TR58	.	.	.	.	.
TR59	.	.	.	.	.
TR60	.	.	.	.	.
TR61	.	.	.	.	.
TR62	.	.	.	.	.
TR63	.	.	.	.	.
TR64	.	.	.	.	.
TR65	.	.	.	.	.
TR66	.	.	.	.	.
TR67	.	.	.	.	.
TR68	.	.	.	.	.
TR69	.	.	.	.	.
TR70	.	.	.	.	.
TR71	.	.	.	.	.
TR72	.	.	.	.	.
TR73	.	.	.	.	.
TR74	.	.	.	.	.
TR75	.	.	.	.	.
TR76	.	.	.	.	.
TR77	.	.	.	.	.
TR78	.	.	.	.	.
TR79	.	.	.	.	.
TR80	.	.	.	.	.
TR81	.	.	.	.	.
TR82	.	.	.	.	.
TW116	0.00000	.	.	.	.
TW117	0.39130	0.00000	.	.	.
TW118	0.47727	0.28947	0.00000	.	.
TW119	0.52174	0.21622	0.26471	0.00000	.
TW120	0.53191	0.23684	0.28571	0.03226	0.00000

APPENDIX C

STRUCTURE Output

STRUCTURE by Pritchard, Stephens and Donnelly (2000)
and Falush, Stephens and Pritchard (2003)
Code by Pritchard and Falush
Version 2.2 (March 2007)

Run parameters:
86 individuals
78 loci
5 populations assumed
50000 Burn-in period
100000 Reps
RECESSIVE ALLELES model used

Overall proportion of membership of the
sample in each of the 5 clusters

Inferred Clusters

1	2	3	4	5
0.148	0.055	0.346	0.183	0.267

[Where 1 = red, 2 = green, 3 =blue, 4 = yellow, and 5 = purple in the graphical output and subsequent explanations in the text.]

Allele-freq. divergence among pops (Net nucleotide distance),
computed using point estimates of P.

	1	2	3	4	5
1	-	0.5297	0.6378	0.7318	0.7333
2	0.5297	-	0.2927	0.3588	0.2549
3	0.6378	0.2927	-	0.3596	0.3254
4	0.7318	0.3588	0.3596	-	0.4097
5	0.7333	0.2549	0.3254	0.4097	-

Average distances (expected heterozygosity) between individuals in same cluster:
cluster 1 : -5.3404
cluster 2 : -4.4254
cluster 3 : -5.3201
cluster 4 : -5.2867
cluster 5 : -5.0908

Estimated Ln Prob of Data = -2062.2
Mean value of ln likelihood = -1665.1
Variance of ln likelihood = 794.1
Mean value of alpha = 0.0570

Mean value of Fst_1 = 0.5940
Mean value of Fst_2 = 0.1881
Mean value of Fst_3 = 0.4148
Mean value of Fst_4 = 0.4921
Mean value of Fst_5 = 0.3043

Inferred ancestry of individuals:

	Label	(%Miss)	:	Inferred clusters				
1	TR1	(0)	:	0.541	0.431	0.015	0.006	0.007
2	TR2	(0)	:	0.458	0.531	0.005	0.003	0.004
3	TR3	(0)	:	0.024	0.746	0.206	0.010	0.013
4	TR4	(0)	:	0.942	0.011	0.020	0.014	0.012
5	TR5	(0)	:	0.963	0.007	0.012	0.009	0.009
6	TR6	(0)	:	0.962	0.007	0.014	0.007	0.009
7	TR7	(0)	:	0.143	0.048	0.695	0.031	0.084
8	TR8	(0)	:	0.934	0.013	0.015	0.029	0.009
9	TR9	(0)	:	0.009	0.009	0.827	0.014	0.141
10	TR10	(0)	:	0.980	0.004	0.008	0.005	0.004
11	TR11	(0)	:	0.929	0.012	0.026	0.016	0.016
12	TR12	(0)	:	0.974	0.005	0.006	0.009	0.006
13	TR13	(0)	:	0.008	0.009	0.955	0.011	0.016
14	TR14	(0)	:	0.969	0.010	0.010	0.005	0.006

15	TR15	(0)	:	0.011	0.008	0.950	0.016	0.016
16	TR16	(0)	:	0.064	0.009	0.899	0.017	0.011
17	TR17	(0)	:	0.015	0.007	0.962	0.008	0.009
18	TR18	(0)	:	0.004	0.005	0.977	0.007	0.006
19	TR20	(0)	:	0.518	0.008	0.453	0.009	0.012
20	TR21	(0)	:	0.007	0.005	0.970	0.007	0.010
21	TR22	(0)	:	0.007	0.017	0.080	0.267	0.629
22	TR23	(0)	:	0.005	0.010	0.717	0.015	0.253
23	TR24	(0)	:	0.208	0.021	0.060	0.031	0.681
24	TR25	(0)	:	0.004	0.006	0.018	0.009	0.963
25	TR26	(0)	:	0.007	0.006	0.008	0.006	0.973
26	TR27	(0)	:	0.179	0.012	0.056	0.013	0.740
27	TR28	(0)	:	0.010	0.008	0.012	0.038	0.931
28	TR29	(0)	:	0.003	0.005	0.011	0.007	0.974
29	TR30	(0)	:	0.015	0.008	0.011	0.010	0.957
30	TR31	(0)	:	0.011	0.017	0.182	0.558	0.232
31	TR32	(0)	:	0.004	0.053	0.013	0.008	0.923
32	TR33	(0)	:	0.012	0.009	0.009	0.023	0.947
33	TR34	(0)	:	0.071	0.012	0.041	0.018	0.859
34	TR35	(0)	:	0.012	0.011	0.020	0.016	0.942
35	TR36	(0)	:	0.011	0.011	0.559	0.012	0.408
36	TR37	(0)	:	0.008	0.011	0.033	0.022	0.927
37	TR38	(0)	:	0.006	0.006	0.013	0.007	0.967
38	TR41	(0)	:	0.027	0.097	0.010	0.848	0.018
39	TR39	(0)	:	0.004	0.005	0.013	0.007	0.971
40	TR40	(0)	:	0.190	0.216	0.423	0.112	0.059
41	TR42	(0)	:	0.016	0.010	0.014	0.011	0.949
42	TR43	(0)	:	0.014	0.011	0.010	0.006	0.959
43	TR44	(0)	:	0.015	0.014	0.150	0.783	0.038
44	TR45	(0)	:	0.004	0.016	0.221	0.682	0.076
45	TR46	(0)	:	0.415	0.010	0.059	0.505	0.012
46	TR47	(0)	:	0.006	0.013	0.810	0.120	0.051
47	TR48	(0)	:	0.008	0.011	0.057	0.808	0.116
48	TR49	(0)	:	0.469	0.016	0.328	0.163	0.024
49	TR50	(0)	:	0.005	0.876	0.035	0.041	0.043

50	TR51	(0)	:	0.004	0.024	0.012	0.008	0.953
51	TR52	(0)	:	0.005	0.013	0.043	0.020	0.919
52	TR53	(0)	:	0.021	0.012	0.011	0.037	0.920
53	TR54	(0)	:	0.045	0.015	0.034	0.739	0.168
54	TR55	(0)	:	0.007	0.014	0.834	0.065	0.080
55	TR56	(0)	:	0.008	0.010	0.903	0.054	0.026
56	TR57	(0)	:	0.004	0.006	0.048	0.920	0.021
57	TR58	(0)	:	0.010	0.013	0.093	0.855	0.030
58	TR59	(0)	:	0.081	0.005	0.006	0.904	0.004
59	TR60	(0)	:	0.009	0.004	0.010	0.972	0.005
60	TR61	(0)	:	0.203	0.006	0.004	0.782	0.005
61	TR62	(0)	:	0.077	0.009	0.011	0.899	0.004
62	TR63	(0)	:	0.003	0.006	0.014	0.969	0.008
63	TR64	(0)	:	0.019	0.005	0.010	0.962	0.005
64	TR65	(0)	:	0.227	0.007	0.006	0.753	0.007
65	TR66	(0)	:	0.018	0.025	0.937	0.011	0.008
66	TR67	(0)	:	0.232	0.011	0.731	0.013	0.013
67	TR68	(0)	:	0.094	0.008	0.882	0.009	0.007
68	TR69	(0)	:	0.018	0.008	0.933	0.030	0.011
69	TR70	(0)	:	0.067	0.010	0.895	0.018	0.011
70	TR71	(0)	:	0.038	0.009	0.918	0.027	0.009
71	TR72	(0)	:	0.009	0.007	0.968	0.008	0.008
72	TR73	(0)	:	0.010	0.015	0.807	0.057	0.111
73	TR74	(0)	:	0.043	0.017	0.852	0.070	0.018
74	TR75	(0)	:	0.005	0.005	0.971	0.010	0.008
75	TR76	(0)	:	0.008	0.008	0.944	0.028	0.012
76	TR77	(0)	:	0.004	0.007	0.912	0.053	0.023
77	TR78	(0)	:	0.004	0.013	0.733	0.007	0.243
78	TR79	(0)	:	0.004	0.019	0.025	0.030	0.921
79	TR80	(0)	:	0.080	0.022	0.029	0.054	0.815
80	TR81	(0)	:	0.007	0.016	0.512	0.040	0.425
81	TR82	(0)	:	0.005	0.006	0.973	0.006	0.009
82	TW116	(0)	:	0.010	0.970	0.004	0.007	0.008
83	TW117	(0)	:	0.166	0.017	0.772	0.023	0.022
84	TW118	(0)	:	0.005	0.011	0.039	0.923	0.022

85	TW119	(0)	:	0.004 0.005 0.962 0.007 0.022
86	TW120	(0)	:	0.004 0.010 0.954 0.006 0.024

Estimated Allele Frequencies in each cluster
First column gives estimated ancestral frequencies

Locus 1 :
2 alleles
0.0% missing data
1 (0.725) 0.959 0.787 0.678 0.748 0.809
0 (0.275) 0.041 0.213 0.322 0.252 0.191

Locus 2 :
2 alleles
0.0% missing data
1 (0.862) 0.980 0.658 0.973 0.971 0.955
0 (0.138) 0.020 0.342 0.027 0.029 0.045

Locus 3 :
2 alleles
0.0% missing data
0 (0.949) 0.962 0.973 0.999 0.998 0.997
1 (0.051) 0.038 0.027 0.001 0.002 0.003

Locus 4 :
2 alleles
0.0% missing data
0 (0.948) 0.999 0.899 0.999 0.998 0.997
1 (0.052) 0.001 0.101 0.001 0.002 0.003

Locus 5 :
2 alleles
0.0% missing data
0 (0.938) 0.998 0.973 0.998 0.313 0.996
1 (0.062) 0.002 0.027 0.002 0.687 0.004

Locus 6 :
2 alleles
0.0% missing data
1 (0.782) 0.958 0.822 0.947 0.048 0.924
0 (0.218) 0.042 0.178 0.053 0.952 0.076

Locus 7 :
2 alleles
0.0% missing data
1 (0.870) 0.978 0.892 0.974 0.631 0.958
0 (0.130) 0.022 0.108 0.026 0.369 0.042

Locus 8 :
2 alleles
0.0% missing data
1 (0.859) 0.978 0.656 0.973 0.970 0.953
0 (0.141) 0.022 0.344 0.027 0.030 0.047

Locus 9 :
2 alleles
0.0% missing data
1 (0.085) 0.519 0.056 0.002 0.031 0.005
0 (0.915) 0.481 0.944 0.998 0.969 0.995

Locus 10 :
2 alleles
0.0% missing data
0 (0.949) 0.996 0.912 0.999 0.998 0.997
1 (0.051) 0.004 0.088 0.001 0.002 0.003

Locus 11 :
2 alleles
0.0% missing data
1 (0.083) 0.003 0.450 0.002 0.003 0.005
0 (0.917) 0.997 0.550 0.998 0.997 0.995

Locus 12 :
2 alleles
0.0% missing data
0 (0.947) 0.996 0.911 0.999 0.998 0.997
1 (0.053) 0.004 0.089 0.001 0.002 0.003

Locus 13 :
2 alleles
0.0% missing data
0 (0.949) 0.996 0.912 0.999 0.998 0.997
1 (0.051) 0.004 0.088 0.001 0.002 0.003

Locus 14 :
2 alleles
0.0% missing data
0 (0.948) 0.996 0.911 0.999 0.999 0.997
1 (0.052) 0.004 0.089 0.001 0.001 0.003

Locus 15 :
2 alleles
0.0% missing data
0 (0.893) 0.997 0.947 0.984 0.976 0.924
1 (0.107) 0.003 0.053 0.016 0.024 0.076

Locus 16 :
2 alleles
0.0% missing data
0 (0.889) 0.814 0.922 0.987 0.979 0.993
1 (0.111) 0.186 0.078 0.013 0.021 0.007

Locus 17 :
2 alleles
0.0% missing data
0 (0.872) 0.997 0.943 0.945 0.995 0.712
1 (0.128) 0.003 0.057 0.055 0.005 0.288

Locus 18 :

2 alleles

0.0% missing data

1 (0.436) 0.941 0.472 0.168 0.191 0.218

0 (0.564) 0.059 0.528 0.832 0.809 0.782

Locus 19 :

2 alleles

0.0% missing data

1 (0.874) 0.358 0.903 0.976 0.976 0.959

0 (0.126) 0.642 0.097 0.024 0.024 0.041

Locus 20 :

2 alleles

0.0% missing data

1 (0.420) 0.916 0.297 0.884 0.310 0.072

0 (0.580) 0.084 0.703 0.116 0.690 0.928

Locus 21 :

2 alleles

0.0% missing data

0 (0.651) 0.946 0.636 0.832 0.224 0.869

1 (0.349) 0.054 0.364 0.168 0.776 0.131

Locus 22 :

2 alleles

0.0% missing data

1 (0.075) 0.003 0.334 0.002 0.002 0.004

0 (0.925) 0.997 0.666 0.998 0.998 0.996

Locus 23 :

2 alleles

0.0% missing data

1 (0.369) 0.937 0.330 0.081 0.286 0.162

0 (0.631) 0.063 0.670 0.919 0.714 0.838

Locus 24 :
2 alleles
0.0% missing data
0 (0.808) 0.992 0.914 0.802 0.933 0.755
1 (0.192) 0.008 0.086 0.198 0.067 0.245

Locus 25 :
2 alleles
0.0% missing data
0 (0.942) 0.999 0.971 0.979 0.998 0.982
1 (0.058) 0.001 0.029 0.021 0.002 0.018

Locus 26 :
2 alleles
0.0% missing data
0 (0.874) 0.996 0.944 0.990 0.964 0.577
1 (0.126) 0.004 0.056 0.010 0.036 0.423

Locus 27 :
2 alleles
0.0% missing data
1 (0.710) 0.637 0.792 0.948 0.221 0.824
0 (0.290) 0.363 0.208 0.052 0.779 0.176

Locus 28 :
2 alleles
0.0% missing data
1 (0.458) 0.219 0.355 0.846 0.462 0.324
0 (0.542) 0.781 0.645 0.154 0.538 0.676

Locus 29 :
2 alleles
0.0% missing data
0 (0.934) 0.998 0.971 0.998 0.623 0.993
1 (0.066) 0.002 0.029 0.002 0.377 0.007

Locus 30 :
2 alleles
0.0% missing data
0 (0.948) 0.994 0.953 0.994 0.996 0.996
1 (0.052) 0.006 0.047 0.006 0.004 0.004

Locus 31 :
2 alleles
0.0% missing data
0 (0.921) 0.998 0.962 0.995 0.992 0.875
1 (0.079) 0.002 0.038 0.005 0.008 0.125

Locus 32 :
2 alleles
0.0% missing data
1 (0.071) 0.842 0.043 0.002 0.009 0.004
0 (0.929) 0.158 0.957 0.998 0.991 0.996

Locus 33 :
2 alleles
0.0% missing data
0 (0.935) 0.998 0.968 0.993 0.998 0.963
1 (0.065) 0.002 0.032 0.007 0.002 0.037

Locus 34 :
2 alleles
0.0% missing data
0 (0.890) 0.782 0.839 0.997 0.984 0.993
1 (0.110) 0.218 0.161 0.003 0.016 0.007

Locus 35 :
2 alleles
0.0% missing data
1 (0.875) 0.979 0.892 0.975 0.972 0.808
0 (0.125) 0.021 0.108 0.025 0.028 0.192

Locus 36 :
2 alleles
0.0% missing data
1 (0.870) 0.978 0.891 0.744 0.973 0.958
0 (0.130) 0.022 0.109 0.256 0.027 0.042

Locus 37 :
2 alleles
0.0% missing data
0 (0.949) 0.998 0.969 0.985 0.998 0.997
1 (0.051) 0.002 0.031 0.015 0.002 0.003

Locus 38 :
2 alleles
0.0% missing data
1 (0.874) 0.981 0.894 0.975 0.973 0.804
0 (0.126) 0.019 0.106 0.025 0.027 0.196

Locus 39 :
2 alleles
0.0% missing data
0 (0.360) 0.646 0.534 0.124 0.066 0.214
1 (0.640) 0.354 0.466 0.876 0.934 0.786

Locus 40 :
2 alleles
0.0% missing data
1 (0.836) 0.975 0.865 0.968 0.968 0.481
0 (0.164) 0.025 0.135 0.032 0.032 0.519

Locus 41 :
2 alleles
0.0% missing data
1 (0.818) 0.972 0.853 0.966 0.961 0.365
0 (0.182) 0.028 0.147 0.034 0.039 0.635

Locus 42 :
2 alleles
0.0% missing data
0 (0.939) 0.999 0.973 0.998 0.998 0.909
1 (0.061) 0.001 0.027 0.002 0.002 0.091

Locus 43 :
2 alleles
0.0% missing data
1 (0.877) 0.982 0.896 0.826 0.975 0.960
0 (0.123) 0.018 0.104 0.174 0.025 0.040

Locus 44 :
2 alleles
0.0% missing data
1 (0.593) 0.916 0.676 0.627 0.647 0.317
0 (0.407) 0.084 0.324 0.373 0.353 0.683

Locus 45 :
2 alleles
0.0% missing data
0 (0.949) 0.999 0.974 0.998 0.998 0.978
1 (0.051) 0.001 0.026 0.002 0.002 0.022

Locus 46 :
2 alleles
0.0% missing data
0 (0.949) 0.999 0.974 0.984 0.998 0.996
1 (0.051) 0.001 0.026 0.016 0.002 0.004

Locus 47 :
2 alleles
0.0% missing data
1 (0.083) 0.003 0.450 0.002 0.003 0.005
0 (0.917) 0.997 0.550 0.998 0.997 0.995

Locus 48 :
2 alleles
0.0% missing data
0 (0.948) 0.999 0.967 0.999 0.998 0.980
1 (0.052) 0.001 0.033 0.001 0.002 0.020

Locus 49 :
2 alleles
0.0% missing data
1 (0.793) 0.904 0.841 0.215 0.959 0.923
0 (0.207) 0.096 0.159 0.785 0.041 0.077

Locus 50 :
2 alleles
0.0% missing data
0 (0.940) 0.998 0.972 0.859 0.998 0.996
1 (0.060) 0.002 0.028 0.141 0.002 0.004

Locus 51 :
2 alleles
0.0% missing data
1 (0.865) 0.942 0.834 0.916 0.949 0.948
0 (0.135) 0.058 0.166 0.084 0.051 0.052

Locus 52 :
2 alleles
0.0% missing data
1 (0.859) 0.979 0.669 0.971 0.968 0.952
0 (0.141) 0.021 0.331 0.029 0.032 0.048

Locus 53 :
2 alleles
0.0% missing data
0 (0.948) 0.999 0.968 0.999 0.998 0.980
1 (0.052) 0.001 0.032 0.001 0.002 0.020

Locus 54 :
2 alleles
0.0% missing data
0 (0.898) 0.997 0.953 0.989 0.996 0.474
1 (0.102) 0.003 0.047 0.011 0.004 0.526

Locus 55 :
2 alleles
0.0% missing data
0 (0.923) 0.998 0.858 0.998 0.981 0.995
1 (0.077) 0.002 0.142 0.002 0.019 0.005

Locus 56 :
2 alleles
0.0% missing data
0 (0.949) 0.996 0.912 0.999 0.999 0.997
1 (0.051) 0.004 0.088 0.001 0.001 0.003

Locus 57 :
2 alleles
0.0% missing data
0 (0.947) 0.999 0.899 0.999 0.998 0.997
1 (0.053) 0.001 0.101 0.001 0.002 0.003

Locus 58 :
2 alleles
0.0% missing data
1 (0.288) 0.797 0.240 0.296 0.017 0.138
0 (0.712) 0.203 0.760 0.704 0.983 0.862

Locus 59 :
2 alleles
0.0% missing data
1 (0.568) 0.601 0.712 0.337 0.178 0.731
0 (0.432) 0.399 0.288 0.663 0.822 0.269

Locus 60 :
2 alleles
0.0% missing data
1 (0.859) 0.979 0.655 0.972 0.969 0.954
0 (0.141) 0.021 0.345 0.028 0.031 0.046

Locus 61 :
2 alleles
0.0% missing data
1 (0.788) 0.969 0.830 0.683 0.771 0.927
0 (0.212) 0.031 0.170 0.317 0.229 0.073

Locus 62 :
2 alleles
0.0% missing data
1 (0.085) 0.003 0.451 0.002 0.003 0.005
0 (0.915) 0.997 0.549 0.998 0.997 0.995

Locus 63 :
2 alleles
0.0% missing data
1 (0.875) 0.952 0.897 0.976 0.777 0.959
0 (0.125) 0.048 0.103 0.024 0.223 0.041

Locus 64 :
2 alleles
0.0% missing data
0 (0.948) 0.999 0.899 0.999 0.998 0.997
1 (0.052) 0.001 0.101 0.001 0.002 0.003

Locus 65 :
2 alleles
0.0% missing data
0 (0.947) 0.999 0.972 0.999 0.998 0.979
1 (0.053) 0.001 0.028 0.001 0.002 0.021

Locus 66 :

2 alleles

0.0% missing data

1 (0.856) 0.977 0.654 0.972 0.970 0.952

0 (0.144) 0.023 0.346 0.028 0.030 0.048

Locus 67 :

2 alleles

0.0% missing data

0 (0.935) 0.998 0.971 0.998 0.998 0.837

1 (0.065) 0.002 0.029 0.002 0.002 0.163

Locus 68 :

2 alleles

0.0% missing data

1 (0.482) 0.872 0.455 0.707 0.695 0.110

0 (0.518) 0.128 0.545 0.293 0.305 0.890

Locus 69 :

2 alleles

0.0% missing data

0 (0.917) 0.998 0.881 0.998 0.997 0.978

1 (0.083) 0.002 0.119 0.002 0.003 0.022

Locus 70 :

2 alleles

0.0% missing data

1 (0.481) 0.940 0.380 0.374 0.574 0.337

0 (0.519) 0.060 0.620 0.626 0.426 0.663

Locus 71 :

2 alleles

0.0% missing data

1 (0.877) 0.982 0.896 0.826 0.973 0.959

0 (0.123) 0.018 0.104 0.174 0.027 0.041

Locus 72 :
2 alleles
0.0% missing data
1 (0.876) 0.358 0.904 0.978 0.977 0.960
0 (0.124) 0.642 0.096 0.022 0.023 0.040

Locus 73 :
2 alleles
0.0% missing data
1 (0.406) 0.932 0.346 0.092 0.334 0.305
0 (0.594) 0.068 0.654 0.908 0.666 0.695

Locus 74 :
2 alleles
0.0% missing data
1 (0.849) 0.316 0.837 0.973 0.972 0.953
0 (0.151) 0.684 0.163 0.027 0.028 0.047

Locus 75 :
2 alleles
0.0% missing data
1 (0.879) 0.709 0.900 0.976 0.975 0.960
0 (0.121) 0.291 0.100 0.024 0.025 0.040

Locus 76 :
2 alleles
0.0% missing data
1 (0.878) 0.452 0.905 0.977 0.977 0.960
0 (0.122) 0.548 0.095 0.023 0.023 0.040

Locus 77 :
2 alleles
0.0% missing data
0 (0.948) 0.999 0.968 0.999 0.998 0.980
1 (0.052) 0.001 0.032 0.001 0.002 0.020

Locus 78 :

2 alleles

0.0% missing data

1 (0.869) 0.980 0.892 0.744 0.973 0.956

0 (0.131) 0.020 0.108 0.256 0.027 0.044

Values of parameters used in structure:

NUMINDS=86, NUMLOCI=78, MISSING=-9, LABEL=1,
POPDATA=0, POPFLAG=0, PHENOTYPE=0, EXTRACOLS=0,
MAXPOPS=5, BURNIN=50000, NUMREPS=100000, USEPOPINFO=0,
INFERALPHA=1, INFERLAMBDA=0, POPSPECIFICLAMBDA=0,
POPALPHAS=0, COMPUTEPROB=1, NOADMIX=0, ADMBURNIN=2500,
UPDATEFREQ=1, PRINTLIKES=0, INTERMEDSAVE=0, PRINTKLD=0,
PRINTNET=0, PRINTLAMBDA=0, ANCESTDIST=0, NUMBOXES=1000,
ANCESTPINT=0.90000, GENSBACK=2, MIGRPRIOR=0.05000,
PRINTQHAT=0, PRINTQSUM=0, ALPHA=1.0000, FREQSCORR=1,
FPRIORMEAN=0.0100, FPRIORSD=0.0500, ONEFST=0, LAMBDA=0.4413,
UNIFPRIORALPHA=1, ALPHAMAX=10.0000, ALPHAPRIORA=1.0000,
ALPHAPRIORB=2.0000, ALPHAPROPSD=0.0250, STARTATPOPINFO=0,
RANDOMIZE=1, LINKAGE=0, METROFREQ=10, REPORTHITRATE=0,
MARKOVPHASE=-1, PHASED=0, PLOIDY=2, PHASEINFO=0

[STRAT parameters]: NUMSIMSTATS=1000, PHENOTYPECOL=-9,
POOLFREQ=10, LOCUSxONLY=0, EMERROR=0.00100,
MISSINGPHENO=-9,

APPENDIX D

Average-days Survival Index Data

Accession Data for Granville Wilt and Black Shank Tests

* Significantly different from Hicks at the 5% level of probability as determined by the Dunnett procedure
^ Significantly different from K346 at the 5% level of probability as determined by the Dunnett procedure

	Per Plant Based Average Day Survival Means			
	Granville Wilt	Black Shank	Black Shank	Black Shank
Entry	Field	Field	Race 0	Race 1
Hicks	64.14^	52.84^	9.75^	10.71^
K436	87.65*	83.95*	25.71*	19.77*
NC 1071	na	na	26.00*	8.29^
TR 19 (Hybrid)	56.85^	93.00*^	26.00*	20.11*
TR 1	40.26*^	84.74*	25.54*	19.42*
TR 2	48.97*^	80.96*	25.21*	16.75
TR 3	40.18*^	84.57*	24.79*	18.46*
TR 4	32.78*^	85.44*	26.00*	21.96*
TR 5	42.94*^	89.91*	25.08*	21.96*
TR 6	40.02*^	91.99*^	26.00*	23.58*
TR 7	47.47*^	91.29*^	25.54*	24.17*
TR 8	33.17*^	84.42*	25.54*	19.54*
TR 9	33.91*^	83.82*	25.54*	20.27*
TR 10	40.39*^	85.25*	26.00*	21.17*
TR 11	41.96*^	86.19*	24.92*	19.33*
TR 12	55.78^	92.49*^	26.00*	23.94*
TR 13	46.41*^	91.04*	26.00*	21.67*
TR 14	37.18*^	91.57*^	25.83*	22.96*
TR 15	31.61*^	90.89*	26.00*	21.21*
TR 16	46.44*^	92.18*^	26.00*	24.75*
TR 17	45.44*^	90.21*	26.00*	20.46*
TR 18	50.09^	90.73*	26.00*	23.13*
TR 20	50.08^	92.67*	26.00*	25.42*
TR 21	48.34*^	92.17*^	26.00*	24.58*
TR 22	37.47*^	87.50*^	26.00*	24.02*
TR 23	33.28*^	90.96*	25.21*	18.63*
TR 24	42.01*^	87.53*	26.00*	19.95*
TR 25	33.46*^	89.53*	25.75*	24.92*
TR 26	34.26*^	90.54*	25.21*	17.38
TR 27	39.70*^	78.32*	24.75*	15.42
TR 28	39.09*^	88.34*	26.00*	23.42*

	Per Plant Based Average Day Survival Means			
Entry	Granville Wilt Field	Black Shank Field	Black Shank Race 0	Black Shank Race 1
TR 29	35.88*^	89.66*	23.88*	14.96
TR 30	34.85*^	88.24*	24.38*	21.33*
TR 31	42.24*^	91.49*^	23.54*	22.38*
TR 32	34.80*^	89.07*	24.54*	16.70
TR 33	38.18*^	87.96*	25.13*	23.46*
TR 34	45.30*^	90.42*	25.42*	24.58*
TR 35	25.71*^	91.76*^	24.29*	19.00*
TR 36	35.61*^	90.52*	25.42*	21.18*
TR 37	38.53*^	85.97*	26.00*	23.33*
TR 38	41.11*^	87.14*	25.21*	15.17
TR 39	38.86*^	90.11*	25.21*	18.03*
TR 40	41.44*^	90.10*	26.00*	22.40*
TR 41	33.97*^	89.99*	25.83*	17.42
TR 42	30.39*^	89.72*	25.38*	16.58
TR 43	36.85*^	92.11*^	25.71*	22.72*
TR 44	38.82*^	91.18*^	25.42*	21.07*
TR 45	37.56*^	91.48*^	25.21*	20.38*
TR 46	30.51*^	87.74*	25.25*	19.21*
TR 47	35.29*^	91.23*^	24.79*	16.42
TR 48	42.20*^	92.82*^	24.92*	23.67*
TR 49	37.72*^	91.78*^	24.59*	18.83*
TR 50	35.78*^	77.03*	23.00*	13.33
TR 51	36.57*^	86.24*	21.33*^	16.25
TR 52	44.22*^	91.43*^	25.21*	22.44*
TR 53	36.18*^	87.31*	23.63*	18.78*
TR 54	35.01*^	92.31*^	26.00*	23.97*
TR 55	31.69*^	91.20*^	25.54*	17.75
TR 56	32.60*^	88.05*	24.00*	14.92
TR 57	35.42*^	91.89*^	24.63*	24.08*
TR 58	30.90*^	90.50*	23.21*	17.79*
TR 59	35.12*^	88.95*	25.71*	23.33*
TR 60	33.17*^	87.38*	25.42*	20.77*
TR 61	37.03*^	89.13*	25.25*	22.17*
TR 62	33.56*^	91.10*	25.71*	22.38*
TR 63	49.41*^	89.52*	22.92*	24.21*
TR 64	43.39*^	90.68*	25.04*	22.00*

Per Plant Based Average Day Survival Means

Entry	Granville Wilt Field	Black Shank Field	Black Shank Race 0	Black Shank Race 1
TR 65	32.97*^	87.49*	26.00*	20.63*
TR 66	38.31*^	85.05*	26.00*	21.25*
TR 67	61.22^	90.84*	25.54*	24.33*
TR 68	43.58*^	90.70*	26.00*	20.34*
TR 69	43.96*^	92.22*^	24.75*	23.54*
TR 70	35.83*^	91.32*^	23.21*	14.28
TR 71	45.55*^	92.27*^	25.71*	19.17*
TR 72	41.28*^	90.62*	24.63*	21.04*
TR 73	37.33*^	91.07*	24.96*	20.63*
TR 74	50.18^	91.42*^	25.54*	21.13*
TR 75	57.35^	91.06*	25.21*	23.21*
TR 76	59.43^	92.29*^	26.00*	22.43*
TR 77	36.89*^	85.31*	21.71*	14.71
TR 78	36.84*^	90.87*	24.75*	23.29*
TR 79	31.73*^	90.76*	26.00*	21.54*
TR 80	60.11^	89.55*	26.00*	23.00*
TR 81	35.04*^	90.86*	25.21*	15.46
TR 82	55.81^	86.15*	24.33*	17.29
TW 116	37.19*^	81.74*	18.75*^	14.98
TW 117	53.94^	91.27*^	25.05*	19.88*
TW 118	49.21*^	86.10*	23.04*	19.88*
TW 119	49.06*^	87.28*	26.00*	18.10*
TW 120	37.61*^	87.32*	24.33*	16.54

BC_2 Black Shank Race 1 Data

Entry	BC_2 Family	Average Day Survival (days)
Hicks		12.75
K326		26.50
K346		29.00
TR50		24.67
627H	TR50	18.75
627K	TR50	27.17
630H	TR50	25.75
630K	TR50	28.67
631H	TR50	13.50
631K	TR50	21.92
633H	TR50	26.25
633K	TR50	27.42
638H	TR50	22.58
638K	TR50	23.92
639K	TR50	25.20
639K	TR50	18.08
640H	TR50	16.58
640K	TR50	26.67
641H	TR50	27.58
641K	TR50	26.25
TR52		29.00
468H	TR52	25.58
468K	TR52	29.00
469H	TR52	29.00
469K	TR52	28.42
474H	TR52	20.00
474K	TR52	26.50
481H	TR52	29.00
481K	TR52	28.67
490H	TR52	21.08
505H	TR52	24.75
521H	TR52	27.58
521K	TR52	28.42
525H	TR52	25.00
525K	TR52	27.75

Entry	BC_2 Family	Average Day Survival (days)
530H	TR52	18.60
530K	TR52	25.33
593H	TR52	24.75
593K	TR52	26.89
TR53		25.75
598H	TR53	18.45
598K	TR53	21.92
601H	TR53	24.42
601K	TR53	21.58
602H	TR53	28.67
602K	TR53	25.58
603K	TR53	24.17
605H	TR53	27.08
605K	TR53	27.42
617H	TR53	22.08
617K	TR53	27.42
621H	TR53	26.08
621K	TR53	27.75
623H	TR53	21.17
623K	TR53	26.67
624H	TR53	19.58
626H	TR53	23.67
TR68		28.67
536H	TR68	18.67
536K	TR68	22.58
537H	TR68	14.25
537K	TR68	20.33
541H	TR68	18.75
541K	TR68	25.58
542H	TR68	29.00
543H	TR68	20.58
543K	TR68	27.17
544H	TR68	20.17
575H	TR68	18.83
575K	TR68	25.67
TR73		28.64
610H	TR73	22.50
610K	TR73	24.55

Entry	BC_2 Family	Average Day Survival (days)
611H	TR73	22.58
611K	TR73	24.67
613H	TR73	20.83
613K	TR73	27.17
614H	TR73	23.42
614K	TR73	28.67
615H	TR73	22.58
615K	TR73	29.00
TR80		29.00
548H	TR80	25.42
548K	TR80	24.20
550H	TR80	25.00
550K	TR80	26.67
553H	TR80	27.70
553K	TR80	26.83
554H	TR80	25.92
554K	TR80	29.00
555H	TR80	27.38
555K	TR80	27.58
556H	TR80	29.00
556K	TR80	27.92
557H	TR80	17.83
557K	TR80	29.00
562H	TR80	26.00
562K	TR80	24.64
596H	TR80	27.17
596K	TR80	29.00
597H	TR80	25.92
597K	TR80	29.00

APPENDIX E

N. rustica Accession Fact Sheets

All morphological values reported within fact sheets are averages calculated from the data collected during the course of this investigation.

TR 1 PI 499162

4384-HHS

This accession was derived from breeding and selection experiments conducted by H. H. Smith during the 1940s. It has a ridged stem and ovate or cordate leaves that are puckered. When grown in the manner common of flue-cured tobacco, it took an average of 60.5 days to flower and reached a height of 154.9 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 31.8 cm long and 28 cm at its widest point. Its flowers are 21.6 mm long and the limb of the corolla is 14.1 mm in diameter. The capsules, which are rotund in shape, are 1.1 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.2% before plants are topped and 3.7% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 1 is a member of Genetic Group 1.

TR 2 PI 499163

4385 L-5-6

This accession was derived from breeding and selection experiments conducted by H. H. Smith during the 1940s. It has a ridged stem and cordate leaves that are puckered. When grown in the manner common of flue-cured tobacco, it took an average of 50 days to flower and reached a height of 146.3 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 30.6 cm long and 28.1 cm at its widest point. Its flowers are 22.6 mm long and the limb of the corolla is 13.3 mm in diameter. The capsules, which are rotund in shape, are 0.9 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 2.0% before plants are topped and 3.8% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 2 is a member of Genetic Group 2.

TR 3 PI 499164

4386 L-5-6

This accession was derived from breeding and selection experiments conducted by H. H. Smith during the 1940s. It has a ridged stem and ovate or cordate leaves that are puckered. When grown in the manner common of flue-cured tobacco, it took an average of 49.5 days to flower and reached a height of 135.9 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 32.2 cm long and 28.0 cm at its widest point. Its flowers are 25.1 mm long and the limb of the corolla is 16.3 mm in diameter. The capsules, which are rotund in shape, are 1.0 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 2.0% before plants are topped and 3.7% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 3 is a member of Genetic Group 2.

TR 4 PI 499165

4390 L-5-2-1

This accession was derived from breeding and selection experiments conducted by H. H. Smith during the 1940s. It has a ridged stem and ovate leaves that are slightly puckered. When grown in the manner common of flue-cured tobacco, it took an average of 53.5 days to flower and reached a height of 122.5 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 30.1 cm long and 24.3 cm at its widest point. Its flowers are 23.6 mm long and the limb of the corolla is 14.5 mm in diameter. The capsules, which are rotund in shape, are 0.9 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.7% before plants are topped and 3.2% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 4 is a member of Genetic Group 1.

TR 5 PI 499166

4398 L-5-2-1

This accession was derived from breeding and selection experiments conducted by H. H. Smith during the 1940s. It has a ridged stem and ovate or cordate leaves that are puckered. When grown in the manner common of flue-cured tobacco, it took an average of 46 days to flower and reached a height of 117.8 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 34.0 cm long and 25.0 cm at its widest point. Its flowers are 22.5 mm long and the limb of the corolla is 14.1 mm in diameter. The capsules, which are rotund in shape, are 0.9 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.0% before plants are topped and 4.5% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 5 is a member of Genetic Group 1.

TR 6 PI 499167

4399 L-5-2-1

This accession was derived from breeding and selection experiments conducted by H. H. Smith during the 1940s. It has a ridged stem and ovate or cordate leaves that are puckered. When grown in the manner common of flue-cured tobacco, it took an average of 57 days to flower and reached a height of 123.3 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 31.1 cm long and 26.5 cm at its widest point. Its flowers are 24.8 mm long and the limb of the corolla is 16.6 mm in diameter. The capsules, which are rotund in shape, are 1.0 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.3% before plants are topped and 4.5% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 6 is a member of Genetic Group 1.

TR 7 PI 499168

43054 L-5-2-1

This accession was derived from breeding and selection experiments conducted by H. H. Smith during the 1940s. It has a ridged stem and ovate or cordate leaves that are puckered. When grown in the manner common of flue-cured tobacco, it took an average of 50 days to flower and reached a height of 142.1 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 30.3 cm long and 23.8 cm at its widest point. Its flowers are 22.8 mm long and the limb of the corolla is 15.3 mm in diameter. The capsules, which are rotund in shape, are 1.0 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.3% before plants are topped and 4.0% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 7 is a member of Genetic Group 3.

TR 8 PI 499169

43101 L-2-1B X L-6-2-1

This accession was derived from breeding and selection experiments conducted by H. H. Smith during the 1940s. It has a ridged stem and cordate leaves that are puckered. When grown in the manner common of flue-cured tobacco, it took an average of 46.5 days to flower and reached a height of 155.9 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 28.8 cm long and 26.3 cm at its widest point. Its flowers are 24.3 mm long and the limb of the corolla is 16.3 mm in diameter. The capsules, which are rotund in shape, are 0.9 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.0% before plants are topped and 3.6% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 8 is a member of Genetic Group 1.

TR 9 PI 499170

43102-1 L-6-2-1 X L-5-2-1B

This accession was derived from breeding and selection experiments conducted by H. H. Smith during the 1940s. It has a ridged stem and cordate leaves that are puckered. When grown in the manner common of flue-cured tobacco, it took an average of 53.5 days to flower and reached a height of 122.7 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 28.8 cm long and 22.3 cm at its widest point. Its flowers are 23.1 mm long and the limb of the corolla is 14.1 mm in diameter. The capsules, which are rotund in shape, are 0.8 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 0.8% before plants are topped and 4.0% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 9 is a member of Genetic Group 3.

TR 10 PI 499171

43103-5 L-5-2-1 X L-6-2-1

This accession was derived from breeding and selection experiments conducted by H. H. Smith during the 1940s. It has a ridged stem and cordate leaves that are puckered. When grown in the manner common of flue-cured tobacco, it took an average of 64 days to flower and reached a height of 138.9 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 31.6 cm long and 27.1 cm at its widest point. Its flowers are 24.1 mm long and the limb of the corolla is 15.4 mm in diameter. The capsules, which are rotund in shape, are 1.0 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.8% before plants are topped and 3.6% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 10 is a member of Genetic Group 1.

TR 11 PI 499172

43104-1 L-5-2-1B X L-6-2-1

This accession was derived from breeding and selection experiments conducted by H. H. Smith during the 1940s. It has a ridged stem and cordate leaves that are puckered. When grown in the manner common of flue-cured tobacco, it took an average of 64 days to flower and reached a height of 131.0 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 28.8 cm long and 23.6 cm at its widest point. Its flowers are 24.6 mm long and the limb of the corolla is 14.9 mm in diameter. The capsules, which are rotund in shape, are 0.9 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.8% before plants are topped and 3.7% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 11 is a member of Genetic Group 1.

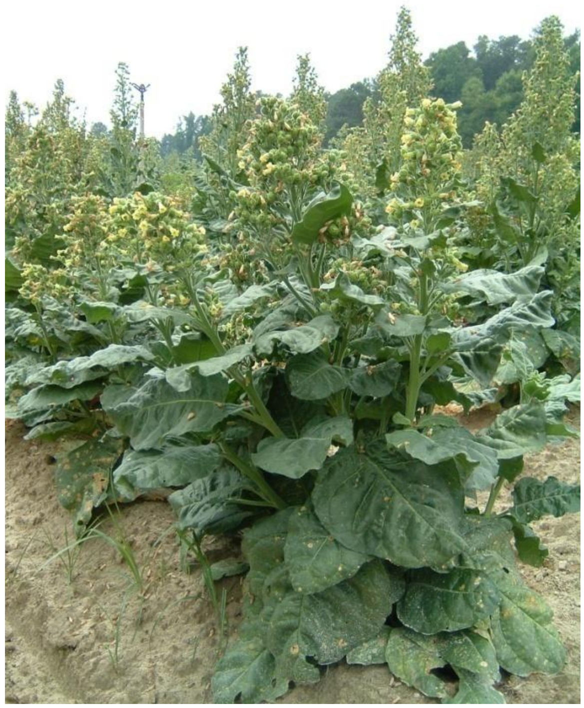
TR 12 PI 499173

4401 L-5-2-1B

This accession was derived from breeding and selection experiments conducted by H. H. Smith during the 1940s. It has a ridged stem and cordate leaves that are puckered. When grown in the manner common of flue-cured tobacco, it took an average of 57 days to flower and reached a height of 117.5 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 30.6 cm long and 26.1 cm at its widest point. Its flowers are 24.8 mm long and the limb of the corolla is 15.6 mm in diameter. The capsules, which are rotund in shape, are 1.0 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.3% before plants are topped and 4.2% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 12 is a member of Genetic Group 1.

TR 13 PI 499174

Brasilia #7



This accession was collected from an unknown location though it was determined to be of the brasilia variety. It has a slightly ridged stem and ovate leaves that are puckered. When grown in the manner common of flue-cured tobacco, it took an average of 53.5 days to flower and reached a height of 78.0 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 24.3 cm long and 21.3 cm at its widest point. Its flowers are 23.6 mm long and the limb of the corolla is 14.3 mm in diameter. The capsules, which are rotund in shape, are 1.1 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.3% before plants are topped and 4.7% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 13 is a member of Genetic Group 3.

TR 14 PI 499175

Brasilia #23

This accession was collected from an unknown location and it was determined to be of the brasilia variety. It has a slightly ridged stem and ovate leaves that are puckered. When grown in the manner common of flue-cured tobacco, it took an average of 47 days to flower and reached a height of 82.0 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 25.5 cm long and 23.5 cm at its widest point. Its flowers are 23.1 mm long and the limb of the corolla is 14.4 mm in diameter. The capsules, which are rotund in shape, are 1.1 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.3% before plants are topped and 4.7% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 14 is a member of Genetic Group 1.

TR 15 PI 34753

Brasilia Selvaggio; *N. rustica* var. *brasilia* Schrank

This accession was collected from the Palmero District of Brazil and it has been reported to be the type strain *N. rustica* var. *brasilia* Schrank. It has ovate leaves that are slightly puckered to puckered. When grown in the manner common of flue-cured tobacco, it took an average of 36.5 days to flower and reached a height of 78.5 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 24.6 cm long and 21.8 cm at its widest point. Its flowers are 22.8 mm long and the limb of the corolla is 15.1 mm in diameter. The capsules, which are rotund in shape, are 1.2 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.0% before plants are topped and 4.5% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 15 is a member of Genetic Group 3.

TR 16 PI 34752

Brasilia Leccese

This accession was collected from the Lecce Province (Nardo District) of Brazil and it was determined to be of an intermediate type with more characteristics of the pumila variety. It has a smooth stem and ovate leaves that are smooth to slightly puckered. When grown in the manner common of flue-cured tobacco, it took an average of 43 days to flower and reached a height of 75.5 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 29.5 cm long and 25.8 cm at its widest point. Its flowers are 21.3 mm long and the limb of the corolla is 13.6mm in diameter. The capsules, which are rotund in shape, are 1.1 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 2.1% before plants are topped and 4.7% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 16 is a member of Genetic Group 3.

TR 17 PI 34754

Erbasanta

This accession was collected from the Lecce Province (Cava dei Terreni District) of Brazil and it was determined to be of an intermediate type with more characteristics of the pumila variety. It has a smooth stem and ovate leaves that are smooth. When grown in the manner common of flue-cured tobacco, it took an average of 53.5 days to flower and reached a height of 80.0 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 22.3 cm long and 21 cm at its widest point. Its flowers are 20.8 mm long and the limb of the corolla is 12.5 mm in diameter. The capsules, which are rotund in shape, are 1.1 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.7% before plants are topped and 5.2% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 17 is a member of Genetic Group 3.

TR 18 PI 499176

68 Olson

This accession is derived from material generated in a cross between the varieties Winnebago and Brasilia #23 (TR 14) by Otto Olson and it was determined to be of the brasilia variety. It has a slightly ridged stem and ovate or cordate leaves that are slightly puckered. When grown in the manner common of flue-cured tobacco, it took an average of 60.5 days to flower and reached a height of 123.9 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 29.1 cm long and 23.0 cm at its widest point. Its flowers are 24.8 mm long and the limb of the corolla is 16.8 mm in diameter. The capsules, which are rotund in shape, are 1.1 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 0.9% before plants are topped and 5.2% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 18 is a member of Genetic Group 3.

TR 20 PI 499178

German #2

This accession was collected from an unknown location and it was determined to be of an intermediate type with more characteristics of the pumila variety. It has a smooth stem and ovate leaves that are slightly puckered. When grown in the manner common of flue-cured tobacco, it took an average of 36 days to flower and reached a height of 66.5 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 19.0 cm long and 17.6 cm at its widest point. Its flowers are 22.8 mm long and the limb of the corolla is 13 mm in diameter. The capsules, which are rotund in shape, are 1 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 2.1% before plants are topped and 6.2% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 20 is a member of Genetic Group 1.

TR 21 PI 499179

German #1

This accession was collected from an unknown location and it was determined to be of an intermediate type with more characteristics of the brasilia variety. It has a smooth stem and ovate leaves that are slightly puckered. When grown in the manner common of flue-cured tobacco, it took an average of 32 days to flower and reached a height of 66.5 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 19.8 cm long and 18.3 cm at its widest point. Its flowers are 23.0 mm long and the limb of the corolla is 15.1 mm in diameter. The capsules, which are rotund in shape, are 1 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.3% before plants are topped and 6.4% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 21 is a member of Genetic Group 3.

TR 22 PI 499180

Mahorka #1 AC 18/7

This accession is from Russia and it was determined to be of the pumila variety. It has a smooth stem and ovate leaves that are slightly puckered. When grown in the manner common of flue-cured tobacco, it took an average of 39 days to flower and reached a height of 62.2 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 18.6 cm long and 16.1 cm at its widest point. Its flowers are 24.1 mm long and the limb of the corolla is 17.6 mm in diameter. The capsules, which are rotund in shape, are 1.1 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.6% before plants are topped and 6.9% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 22 is a member of Genetic Group 5.

TR 23 PI 499181

Mahorka #2 Armenia

This accession is from Russia and it was determined to be of the brasilia variety. It has a smooth stem and ovate or cordate leaves that are slightly puckered to puckered. When grown in the manner common of flue-cured tobacco, it took an average of 43 days to flower and reached a height of 99.5 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 29.5 cm long and 26.3 cm at its widest point. Its flowers are 24.6 mm long and the limb of the corolla is 16.8 mm in diameter. The capsules, which are rotund in shape, are 1.1 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.7% before plants are topped and 4.7% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 23 is a member of Genetic Group 3.

TR 24 PI 499182

Mahorka #3 Voronezhskaia

This accession is from Russia and it was determined to be of the pumila variety. It has a smooth stem and ovate leaves that are smooth to slightly puckered. When grown in the manner common of flue-cured tobacco, it took an average of 36 days to flower and reached a height of 77.5 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 16.8 cm long and 13.6 cm at its widest point. Its flowers are 24.8 mm long and the limb of the corolla is 17.6 mm in diameter. The capsules, which are rotund in shape, are 1.0 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 0.9% before plants are topped and 3.7% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 24 is a member of Genetic Group 5.

TR 25 PI 499183

Mahorka #4 Tall Green

This accession is from Russia and it was determined to be of the pumila variety. It has a smooth stem and ovate or cordate leaves that are slightly puckered. When grown in the manner common of flue-cured tobacco, it took an average of 36 days to flower and reached a height of 69.2 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 18.8 cm long and 16.5 cm at its widest point. Its flowers are 25.5 mm long and the limb of the corolla is 19.6 mm in diameter. The capsules, which are rotund in shape, are 1.2 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.1% before plants are topped and 2.8% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 25 is a member of Genetic Group 5.

TR 26 PI 499184

Mahorka #5

This accession is from Russia and it was determined to be of an intermediate type. It has a smooth stem and ovate leaves that are puckered. When grown in the manner common of flue-cured tobacco, it took an average of 36 days to flower and reached a height of 64.7 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 19.6 cm long and 15.9 cm at its widest point. Its flowers are 25 mm long and the limb of the corolla is 19.6 mm in diameter. The capsules, which are rotund in shape, are 1.1 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.2% before plants are topped and 3.2% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 26 is a member of Genetic Group 5.

TR 27 PI 499185

Mahorka #6 Yellow #109

This accession is from Russia and it was determined to be of an intermediate type. It has a smooth white stem and ovate leaves that are yellow-green and slightly puckered. In contrast to the typical dark green of *N. rustica*, this unique coloring has been described as aurea or burley type. When grown in the manner common of flue-cured tobacco, it took an average of 32 days to flower and reached a height of 65.7 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 18.6 cm long and 17.6 cm at its widest point. Its flowers are 24.6 mm long and the limb of the corolla is 17.6 mm in diameter. The capsules, which are rotund in shape, are 1.1 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 0.6% before plants are topped and 2.5% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 27 is a member of Genetic Group 5.

TR 28 PI 499186

Mahorka #7 Pekhletzenskaia

This accession is from Russia and it was determined to be of the pumila variety. It has a smooth stem and ovate leaves that are slightly puckered. When grown in the manner common of flue-cured tobacco, it took an average of 32 days to flower and reached a height of 70.9 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 14.4 cm long and 11.5 cm at its widest point. Its flowers are 25.3 mm long and the limb of the corolla is 17.3 mm in diameter. The capsules, which are rotund in shape, are 1.2 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.5% before plants are topped and 4.0% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 28 is a member of Genetic Group 5.

TR 29 PI 499187

Mahorka #8 Prosechenskaia Local

This accession is from Russia and it was determined to be of an intermediate type. It has a smooth stem and cordate leaves that are slightly puckered. When grown in the manner common of flue-cured tobacco, it took an average of 53.5 days to flower and reached a height of 66.0 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 23.3 cm long and 23.3 cm at its widest point. Its flowers are 24.5 mm long and the limb of the corolla is 15.9 mm in diameter. The capsules, which are rotund in shape, are 1 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 2.1% before plants are topped and 3.7% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 29 is a member of Genetic Group 5.

TR 30 PI 499188

Mahorka #9 Stapukhenskaia

This accession is from Russia and it was determined to be of an intermediate type. It has a ridged stem and cordate leaves that are puckered. When grown in the manner common of flue-cured tobacco, it took an average of 39 days to flower and reached a height of 62.2 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 23.5 cm long and 22.6 cm at its widest point. Its flowers are 26.1 mm long and the limb of the corolla is 17.3 mm in diameter. The capsules, which are rotund in shape, are 1.2 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.3% before plants are topped and 3.2% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 30 is a member of Genetic Group 5.

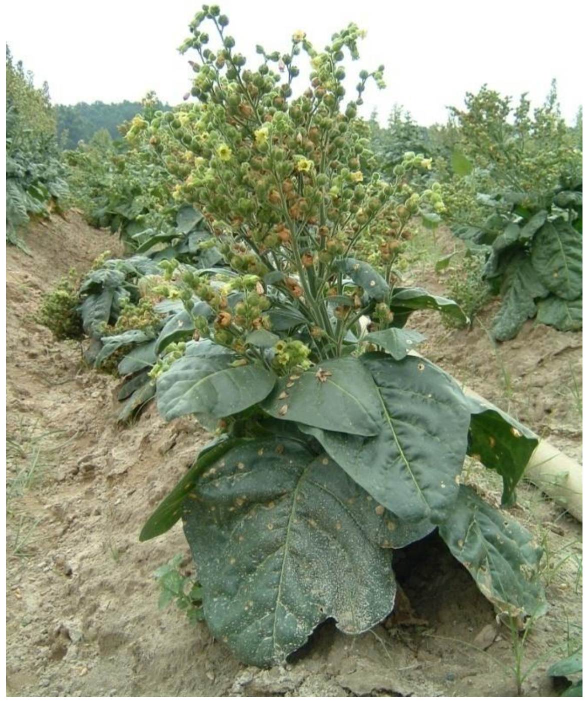
TR 31 PI 499189

Mahorka #10 Sorotooskaia

This accession is from Russia and it was determined to be of the brasilia variety. It has a slightly ridged stem and ovate or cordate leaves that are puckered. When grown in the manner common of flue-cured tobacco, it took an average of 43 days to flower and reached a height of 64.9 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 26.8 cm long and 26.1 cm at its widest point. Its flowers are 25.3 mm long and the limb of the corolla is 17.0 mm in diameter. The capsules, which are rotund in shape, are 1.2 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.8% before plants are topped and 3.8% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 31 is a member of Genetic Group 4.

TR 32 PI 499190

Mahorka #11 Stalingradskaia



This accession is from Russia and it was determined to be of an intermediate type with more characteristics of the brasilia variety. It has a smooth stem and ovate leaves that are smooth to slightly puckered. When grown in the manner common of flue-cured tobacco, it took an average of 32 days to flower and reached a height of 66.0 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 23.1 cm long and 21.6 cm at its widest point. Its flowers are 24.8 mm long and the limb of the corolla is 17.1 mm in diameter. The capsules, which are rotund in shape, are 1.2 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.3% before plants are topped and 3.0% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 32 is a member of Genetic Group 5.

TR 33 PI 499191

Mahorka #12 Ivisenskaia Local

This accession is from Russia and it was determined to be of the pumila variety. It has a smooth stem and ovate leaves that are smooth. When grown in the manner common of flue-cured tobacco, it took an average of 28 days to flower and reached a height of 68.0 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 15.8 cm long and 12.1 cm at its widest point. Its flowers are 24.5 mm long and the limb of the corolla is 16.1 mm in diameter. The capsules, which are rotund in shape, are 1.0 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.1% before plants are topped and 4.5% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 33 is a member of Genetic Group 5.

TR 34 PI 499192

Kostoff White Seed #14

This accession is from Russia and it was determined to be of the pumila variety. It has a smooth stem and ovate leaves that are smooth. When grown in the manner common of flue-cured tobacco, it took an average of 39 days to flower and reached a height of 72.0 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 17.8 cm long and 14.6 cm at its widest point. Its flowers are 24.3 mm long and the limb of the corolla is 16.6 mm in diameter. The capsules, which are rotund in shape, are 1.1 cm in diameter. This accession has unique white seed coats, rather than brown. The percent total alkaloids, which is primarily nicotine, is 1.1% before plants are topped and 4.0% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 34 is a member of Genetic Group 5.

TR 35 PI 499193

BAK #46

This accession is from Russia and it was determined to be of the brasilia variety. It has a smooth stem and ovate leaves that are slightly puckered to puckered. When grown in the manner common of flue-cured tobacco, it took an average of 46.5 days to flower and reached a height of 77.0 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 28.8 cm long and 27.6 cm at its widest point. Its flowers are 25.3 mm long and the limb of the corolla is 17.6 mm in diameter. The capsules, which are rotund in shape, are 1.2 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 0.8% before plants are topped and 2.5% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 35 is a member of Genetic Group 5.

TR 36 PI 499194

Koriotes Dark-blue

This accession is from Russia and it was determined to be of the brasilia variety. It has a smooth stem and ovate or cordate leaves that are slightly puckered to puckered. When grown in the manner common of flue-cured tobacco, it took an average of 35.5 days to flower and reached a height of 84.2 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 26.6 cm long and 25.8 cm at its widest point. Its flowers are 26.1 mm long and the limb of the corolla is 18.1 mm in diameter. The capsules, which are rotund in shape, are 1.2 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.7% before plants are topped and 3.7% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 36 is a member of Genetic Group 3.

TR 37 PI 499195

Jainkaya Soldata

This accession is from Russia and it was determined to be of the pumila variety. It has a smooth stem and ovate leaves that are smooth. When grown in the manner common of flue-cured tobacco, it took an average of 28 days to flower and reached a height of 51.0 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 14.4 cm long and 10.5 cm at its widest point. Its flowers are 26.8 mm long and the limb of the corolla is 19.6 mm in diameter. The capsules, which are rotund in shape, are 1.1 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.0% before plants are topped and 4.4% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 37 is a member of Genetic Group 5.

TR 38 PI 499196

Jainkaya Black-blue #54

This accession is from Russia and it was determined to be of the pumila variety. It has a smooth stem and broad elliptic or ovate leaves that are smooth to slightly puckered. When grown in the manner common of flue-cured tobacco, it took an average of 32 days to flower and reached a height of 67.7 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 17.1 cm long and 13.1 cm at its widest point. Its flowers are 25.8 mm long and the limb of the corolla is 19.1 mm in diameter. The capsules, which are rotund in shape, are 1.1 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.5% before plants are topped and 3.6% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 38 is a member of Genetic Group 5.

TR 39 PI 499197

Drosgi Black-blue #45

This accession is from Russia and it was determined to be of the pumila variety. It has a smooth stem and ovate leaves that are smooth. When grown in the manner common of flue-cured tobacco, it took an average of 53.5 days to flower and reached a height of 73.5 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 13.5 cm long and 10.5 cm at its widest point. Its flowers are 24.1 mm long and the limb of the corolla is 16.1 mm in diameter. The capsules, which are rotund in shape, are 1.0 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.3% before plants are topped and 3.5% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 39 is a member of Genetic Group 5.

TR 40 PI 499198

14 NO. 23057

This accession was collected from Russia and it was determined to be of the pumila variety. It has a smooth stem and elliptic leaves that are smooth. When grown in the manner common of flue-cured tobacco, it took an average of 36 days to flower and reached a height of 74.5 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 15.0 cm long and 9.0 cm at its widest point. Its flowers are 23.8 mm long and the limb of the corolla is 15.6 mm in diameter. The capsules, which are rotund in shape, are quite small though they are 0.9 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.3% before plants are topped and 3.1% two weeks after topping, but may reach much higher levels when heavily fertilized. The genetic group of TR 40 is not defined.

TR 41 PI 499199

Edinburg #25

This accession is from Russia and it was determined to be an intermediate type, sharing characteristics of both the pumila and pavonii varieties. It has a smooth stem and broad elliptic or ovate leaves that are smooth. When grown in the manner common of flue-cured tobacco, it took an average of 32 days to flower and reached a height of 86.0 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 17.3 cm long and 12.9 cm at its widest point. Its flowers are 23.8 mm long and the limb of the corolla is 16.3 mm in diameter. The capsules, which are elliptical in shape, are 0.9 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.3% before plants are topped and 3.7% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 41 is a member of Genetic Group 4.

TR 42 PI 499200

JA. BOT. CAR. #30

This accession is from a collection in France and it was determined to be of the pumila variety. It has a smooth stem and broad elliptic or ovate leaves that are smooth. When grown in the manner common of flue-cured tobacco, it took an average of 28 days to flower and reached a height of 60.7 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 14.1cm long and 10.8 cm at its widest point. Its flowers are 23.6 mm long and the limb of the corolla is 15.8 mm in diameter. The capsules, which are rotund in shape, are 0.9 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.3% before plants are topped and 4.2% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 42 is a member of Genetic Group 5.

TR 43 PI 499201

R. BOT. CAR. #29

This accession is from a collection in Lithuania and it was determined to be of the pumila variety. It has a smooth stem and ovate leaves that are smooth. When grown in the manner common of flue-cured tobacco, it took an average of 35.5 days to flower and reached a height of 65.0 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 15.3 cm long and 12.9 cm at its widest point. Its flowers are 24.8 mm long and the limb of the corolla is 15.8 mm in diameter. The capsules, which are rotund in shape, are 1.0 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.5% before plants are topped and 2.6% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 43 is a member of Genetic Group 5.

TR 44 PI 499202

Harbin #6

This accession is from China and it was determined to be of an intermediate type with more characteristics of the brasilia variety. It has a smooth stem and broad elliptic or ovate leaves that are smooth to slightly puckered. When grown in the manner common of flue-cured tobacco, it took an average of 43 days to flower and reached a height of 74.5 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 27.3 cm long and 22.3 cm at its widest point. Its flowers are 24.1 mm long and the limb of the corolla is 15.4 mm in diameter. The capsules, which are rotund in shape, are 1.2 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.1% before plants are topped and 2.6% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 44 is a member of Genetic Group 4.

TR 45 PI 269933

Normal

This accession was collected from Pakistan and it was determined to be of an intermediate type with more characteristics of the pumila variety. It has a smooth stem and ovate leaves that are smooth. When grown in the manner common of flue-cured tobacco, it took an average of 39.5 days to flower and reached a height of 84.5 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 23.6 cm long and 21.6 cm at its widest point. Its flowers are 22.8 mm long and the limb of the corolla is 15.9 mm in diameter. The capsules, which are rotund in shape, are 1.2 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.3% before plants are topped and 3.3% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 45 is a member of Genetic Group 4.

TR 46 PI 269934

Matsuj Field

This accession was collected from Pakistan and it was determined to be of the brasilia variety. It has a ridged stem and ovate or cordate leaves that are slightly puckered. When grown in the manner common of flue-cured tobacco, it took an average of 36 days to flower and reached a height of 71.9 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 22.8 cm long and 22.5 cm at its widest point. Its flowers are 23.8 mm long and the limb of the corolla is 16.6 mm in diameter. The capsules, which are rotund in shape, are 1.1 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.6% before plants are topped and 4.0% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 46 is a member of Genetic Group 4.

TR 47 PI 269935

Buni Field

This accession was collected from Pakistan and it was determined to be of the brasilia variety. It has a smooth stem and ovate leaves that are slightly puckered. When grown in the manner common of flue-cured tobacco, it took an average of 43 days to flower and reached a height of 81.2 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 23.3 cm long and 20.6 cm at its widest point. Its flowers are 23.3 mm long and the limb of the corolla is 15.6 mm in diameter. The capsules, which are rotund in shape, are 1.1 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.3% before plants are topped and 4.5% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 47 is a member of Genetic Group 3.

TR 48 PI 269936

Dumont

This accession was collected from Pakistan and it was determined to be of an intermediate type. It has a smooth stem and ovate leaves that are smooth. When grown in the manner common of flue-cured tobacco, it took an average of 50 days to flower and reached a height of 94.5 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 26.8 cm long and 24.8 cm at its widest point. Its flowers are 22.1 mm long and the limb of the corolla is 13.9 mm in diameter. The capsules, which are rotund in shape, are 1.1 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.3% before plants are topped and 3.2% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 48 is a member of Genetic Group 4.

TR 49 PI 499203

Chinensis

This accession was collected from Turkey and it was determined to be of the brasilia variety. It has a ridged stem and cordate leaves that are slightly puckered to puckered. When grown in the manner common of flue-cured tobacco, it took an average of 57 days to flower and reached a height of 88.7 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 29.1 cm long and 28.5 cm at its widest point. Its flowers are 23.3 mm long and the limb of the corolla is 15.4 mm in diameter. The capsules, which are rotund in shape, are 1.1 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.8% before plants are topped and 3.2% two weeks after topping, but may reach much higher levels when heavily fertilized. The genetic group of TR 49 is not defined.

TR 50 PI 499204

Campanulata

This accession was collected from New Zealand and it was determined to be of the pumila variety. It has a smooth stem and broad elliptic or ovate leaves that are smooth. When grown in the manner common of flue-cured tobacco, it took an average of 28 days to flower and reached a height of 84.2 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 14.6 cm long and 10.3 cm at its widest point. Its flowers are 21.8 mm long and the limb of the corolla is 12.5 mm in diameter. The capsules, though they are 1.0 cm in diameter, tend to be very small compared to the other accessions and exhibit anthocyanin expression. The percent total alkaloids is very low, on average 1.8% before plants are topped and 1.0% two weeks after topping. This species was once classified as its own species, *N. campanulata*, but was later determined to be a type of *N. rustica*. TR 50 is a member of Genetic Group 2.

TR 51 PI 499205

Acutiflora

This accession was collected from New Zealand and it was determined to be of the pumila variety. It has a smooth stem and broad elliptic or ovate leaves that are smooth. When grown in the manner common of flue-cured tobacco, it took an average of 28 days to flower and reached a height of 53.3 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 13.9 cm long and 11.6 cm at its widest point. Its flowers are 23.0 mm long and the limb of the corolla is 14.8 mm in diameter. The capsules, which are rotund in shape, are 1.1 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.3% before plants are topped and 3.3% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 51 is a member of Genetic Group 5.

TR 52 PI 499206

Fructicora

This accession is from Matueka, New Zealand and it was determined to be of the pumila variety. It has a smooth stem and ovate leaves that are smooth. When grown in the manner common of flue-cured tobacco, it took an average of 28 days to flower and reached a height of 66.7 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 16.5 cm long and 13.0 cm at its widest point. Its flowers are 22.8 mm long and the limb of the corolla is 15.1 mm in diameter. The capsules, which are rotund in shape, are 1.0 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.5% before plants are topped and 4.2% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 52 is a member of Genetic Group 5.

TR 53 PI 499207

Acutifolia

This accession was collected from New Zealand and it was determined to be of the pumila variety. It has a smooth stem and ovate leaves that are smooth. When grown in the manner common of flue-cured tobacco, it took an average of 32 days to flower and reached a height of 62.2 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 14.8 cm long and 13.1 cm at its widest point. Its flowers are 23.3 mm long and the limb of the corolla is 14.6 mm in diameter. The capsules, which are rotund in shape, are 1.0 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.3% before plants are topped and 3.2% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 53 is a member of Genetic Group 5.

TR 54 PI 240355

Nordugel

This accession was collected from New Guinea and it was determined to be of an intermediate type with more characteristics of the brasilia variety. It has a smooth stem and ovate leaves that are slightly puckered. When grown in the manner common of flue-cured tobacco, it took an average of 43 days to flower and reached a height of 75.5 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 24.6 cm long and 19.0 cm at its widest point. Its flowers are 25.3 mm long and the limb of the corolla is 16.6 mm in diameter. The capsules, which are rotund in shape, are 1.1 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.8% before plants are topped and 3.8% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 54 is a member of Genetic Group 4.

TR 55 PI 478886

GC-1

This accession is from India and it was determined to be of the brasilia variety. It has a slightly ridged stem and cordate leaves that are puckered. When grown in the manner common of flue-cured tobacco, it took an average of 43 days to flower and reached a height of 69.9 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 27.8 cm long and 30.0 cm at its widest point. Its flowers are 24.8 mm long and the limb of the corolla is 17.3 mm in diameter. The capsules, which are rotund in shape, are 1.1 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.7% before plants are topped and 3.2% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 55 is a member of Genetic Group 3.

TR 56 PI 481453

Hasankeyf

This accession was collected from Turkey and it was determined to be of an intermediate type with more characteristics of pumila variety. It has a smooth stem and broad elliptic or ovate leaves that are smooth. When grown in the manner common of flue-cured tobacco, it took an average of 39.5 days to flower and reached a height of 79.5 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 22.6 cm long and 19.8 cm at its widest point. Its flowers are 24.1 mm long and the limb of the corolla is 15.9 mm in diameter. The capsules, which are rotund in shape, are 1.2 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.1% before plants are topped and 6.0% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 56 is a member of Genetic Group 3.

TR 57 PI 475761

PNE 241-5

This accession was collected from Raigora, Pakistan and it was determined to be of an intermediate type with more characteristics of the brasilia variety. It has a smooth stem and ovate leaves that are smooth to slightly puckered. When grown in the manner common of flue-cured tobacco, it took an average of 46.5 days to flower and reached a height of 95.7 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 24.0 cm long and 22.6 cm at its widest point. Its flowers are 24.1 mm long and the limb of the corolla is 14.8 mm in diameter. The capsules, which are rotund in shape, are 1.2 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.6% before plants are topped and 3.2% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 57 is a member of Genetic Group 4.

TR 58 PI 475762

PNE 362-4

This accession was collected from Sabura, Pakistan and it was determined to be of the intermediate type with more characteristics of brasilia variety. It has a slightly ridged stem and ovate leaves that are slightly puckered. When grown in the manner common of flue-cured tobacco, it took an average of 50 days to flower and reached a height of 83.7 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 22.8 cm long and 20.8 cm at its widest point. Its flowers are 23.5 mm long and the limb of the corolla is 15.0 mm in diameter. The capsules, which are rotund in shape, are 1.1 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.0% before plants are topped and 3.6% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 58 is a member of Genetic Group 4.

TR 59 PI 475763

PNE 369-3

This accession was collected from Qila Viala, Pakistan and it was determined to be of an intermediate type with more characteristics of the pumila variety. It has a smooth stem and cordate leaves that are slightly puckered. When grown in the manner common of flue-cured tobacco, it took an average of 57 days to flower and reached a height of 79.5 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 26.5 cm long and 25.1 cm at its widest point. Its flowers are 24.8 mm long and the limb of the corolla is 16.3 mm in diameter. The capsules, which are rotund in shape, are 0.9 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.0% before plants are topped and 3.6% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 59 is a member of Genetic Group 4.

TR 60 PI 475764

PNE 373-13

This accession was collected from the Kili Bataizai Kona Bazar, Pakistan and it was determined to be of the brasilia variety. It has a ridged stem and ovate or cordate leaves that are smooth to slightly puckered. When grown in the manner common of flue-cured tobacco, it took an average of 43 days to flower and reached a height of 84.0 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 26.6 cm long and 25.5 cm at its widest point. Its flowers are 24.1 mm long and the limb of the corolla is 16.0 mm in diameter. The capsules, which are rotund in shape, are 1.1 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.7% before plants are topped and 4.0% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 60 is a member of Genetic Group 4.

TR 61 PI 475765

PNE 407-5

This accession was collected from Dilisor, Pakistan and it was determined to be of the pumila variety. It has a smooth stem and ovate or cordate leaves that are slightly puckered. When grown in the manner common of flue-cured tobacco, it took an average of 36 days to flower and reached a height of 79.9 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 24.8 cm long and 22.5 cm at its widest point. Its flowers are 23.8 mm long and the limb of the corolla is 14.9 mm in diameter. The capsules, which are rotund in shape, are 1.1 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.8% before plants are topped and 3.0% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 61 is a member of Genetic Group 4.

TR 62 PI 475766

PNE 412-8

This accession was collected from Babu Cheena, Pakistan and it was determined to be of the pumila variety. It has a smooth or slightly ridged stem and ovate leaves that are slightly puckered. When grown in the manner common of flue-cured tobacco, it took an average of 43 days to flower and reached a height of 95.7 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 24.8 cm long and 23.0 cm at its widest point. Its flowers are 23.6 mm long and the limb of the corolla is 14.9 mm in diameter. The capsules, which are rotund in shape, are 1.2 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.8% before plants are topped and 3.2% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 62 is a member of Genetic Group 4.

TR 63 PI 475767

PNE 417-4

This accession was collected from Watterzion, Pakistan and it was determined to be of the brasilia variety. It has a ridged stem and cordate leaves that are puckered. When grown in the manner common of flue-cured tobacco, it took an average of 50 days to flower and reached a height of 102.8 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 26.8 cm long and 25.5 cm at its widest point. Its flowers are 24.1 mm long and the limb of the corolla is 15.9 mm in diameter. The capsules, which are rotund in shape, are 1.3 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.5% before plants are topped and 3.2% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 63 is a member of Genetic Group 4.

TR 64 PI 475768

PNE 418-6

This accession was collected from Akhtarzai, Pakistan and it was determined to be of the brasilia variety. It has a smooth stem and ovate or cordate leaves that are slightly puckered. When grown in the manner common of flue-cured tobacco, it took an average of 50 days to flower and reached a height of 92.2 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 24.8 cm long and 22.6 cm at its widest point. Its flowers are 23.3 mm long and the limb of the corolla is 15.1 mm in diameter. The capsules, which are rotund in shape, are 1.2 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.5% before plants are topped and 3.5% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 64 is a member of Genetic Group 4.

TR 65 PI 475769

PNE 420-6

This accession was collected from Allozai, Pakistan and it was determined to be of the brasilia variety. It has a ridged stem and cordate leaves that are slightly puckered to puckered. When grown in the manner common of flue-cured tobacco, it took an average of 50 days to flower and reached a height of 116.5 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 27.8 cm long and 27.6 cm at its widest point. Its flowers are 22.3 mm long and the limb of the corolla is 14.3 mm in diameter. The capsules, which are rotund in shape, are 1.2 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.0% before plants are topped and 1.8% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 65 is a member of Genetic Group 4.

TR 66 PI 475770

PNE 427-4

This accession was collected from Zargoon, Pakistan and it was determined to be of the brasilia variety. It has a smooth stem and ovate or cordate leaves that are slightly puckered. When grown in the manner common of flue-cured tobacco, it took an average of 43 days to flower and reached a height of 70.5 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 25.3 cm long and 24.3 cm at its widest point. Its flowers are 22.3 mm long and the limb of the corolla is 13.6 mm in diameter. The capsules, which are rotund in shape, are 1.1 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 0.8% before plants are topped and 3.5% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 66 is a member of Genetic Group 3.

TR 67 PI 494878

ZFA 3544

This accession was collected from the Northern Province of Zambia and it was determined to be of an intermediate type. It has a slightly ridged stem and ovate leaves that are slightly puckered. When grown in the manner common of flue-cured tobacco, it took an average of 46.5 days to flower and reached a height of 86.0 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 24.1 cm long and 19.5 cm at its widest point. Its flowers are 21.3 mm long and the limb of the corolla is 12.4 mm in diameter. The capsules, which are rotund in shape, are 1.0 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.1% before plants are topped and 3.6% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 67 is a member of Genetic Group 3.

TR 68 PI 494879

ZFA 3561

This accession was collected from the Luapula Province of Zambia and it was determined to be of the brasilia variety. It has a smooth stem and ovate leaves that are slightly puckered. When grown in the manner common of flue-cured tobacco, it took an average of 57 days to flower and reached a height of 91.7 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 22.6 cm long and 19.6 cm at its widest point. Its flowers are 21.6 mm long and the limb of the corolla is 12.8 mm in diameter. The capsules, which are rotund in shape, are 1.0 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.3% before plants are topped and 4.2% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 68 is a member of Genetic Group 3.

TR 69 PI 494880

ZFA 3564, Katete

This accession was collected from the Luapula Province of Zambia and it was determined to be of an intermediate type with more characteristics of the pumila variety. It has a smooth stem and ovate leaves that are slightly puckered. When grown in the manner common of flue-cured tobacco, it took an average of 60.5 days to flower and reached a height of 85.7 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 21.6 cm long and 18.8 cm at its widest point. Its flowers are 22.3 mm long and the limb of the corolla is 13.6 mm in diameter. The capsules, which are rotund in shape, are 1.0 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.3% before plants are topped and 4.5% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 69 is a member of Genetic Group 3.

TR 70 PI 500927

This accession was collected from the Western Province of Zambia and it was determined to be of the pumila variety. It has a smooth stem and ovate leaves that are slightly puckered. When grown in the manner common of flue-cured tobacco, it took an average of 36 days to flower and reached a height of 62.7 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 17.6 cm long and 14.8 cm at its widest point. Its flowers are 24.1 mm long and the limb of the corolla is 16.3 mm in diameter. The capsules, which are rotund in shape, are 1.1 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.8% before plants are topped and 3.8% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 70 is a member of Genetic Group 3.

TR 71 PI 500931

This accession was collected from the Luapula Province of Zambia and it was determined to be of an intermediate type. It has a smooth stem and ovate leaves that are slightly puckered. When grown in the manner common of flue-cured tobacco, it took an average of 46.5 days to flower and reached a height of 84.7 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 21.1 cm long and 19.5 cm at its widest point. Its flowers are 24.0 mm long and the limb of the corolla is 15.3 mm in diameter. The capsules, which are rotund in shape, are 1.1 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.3% before plants are topped and 3.5% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 71 is a member of Genetic Group 3.

TR 72 PI 500932

This accession was collected from the Luapula Province of Zambia and it was determined to be of the brasilia variety. It has a smooth stem and ovate leaves that are slightly puckered-puckered. When grown in the manner common of flue-cured tobacco, it took an average of 57 days to flower and reached a height of 90.7 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 23.3 cm long and 22.0 cm at its widest point. Its flowers are 23.8 mm long and the limb of the corolla is 15.3 mm in diameter. The capsules, which are rotund in shape, are 1.0 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.6% before plants are topped and 3.7% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 72 is a member of Genetic Group 3.

TR 73 PI 500933

This accession was collected from the Luapula Province of Zambia and it was determined to be of the brasilia variety. It has a smooth stem and ovate leaves that are slightly puckered. When grown in the manner common of flue-cured tobacco, it took an average of 57 days to flower and reached a height of 85.0 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 25.1 cm long and 22.1 cm at its widest point. Its flowers are 22.6 mm long and the limb of the corolla is 14.4 mm in diameter. The capsules, which are rotund in shape, are 1.0 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.3% before plants are topped and 5.4% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 73 is a member of Genetic Group 3.

TR 74 PI 500936

This accession was collected from the Luapula Province of Zambia and it was determined to be of an intermediate type. It has a smooth stem and ovate leaves that are smooth to slightly puckered. When grown in the manner common of flue-cured tobacco, it took an average of 57 days to flower and reached a height of 90.09 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 24.8 cm long and 19.8 cm at its widest point. Its flowers are 22.3 mm long and the limb of the corolla is 14.1 mm in diameter. The capsules, which are rotund in shape, are 1.1 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.6% before plants are topped and 4.7% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 74 is a member of Genetic Group 3.

TR 75 PI 505664

ZM/A5133

This accession was collected from the Northwestern Province of Zambia and it was determined to be of an intermediate type. It has a slightly ridged stem and ovate leaves that are slightly puckered. When grown in the manner common of flue-cured tobacco, it took an average of 53.5 days to flower and reached a height of 90.5 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 25.1 cm long and 24.0 cm at its widest point. Its flowers are 23.3 mm long and the limb of the corolla is 14.4 mm in diameter. The capsules, which are rotund in shape, are 1.1 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.3% before plants are topped and 5.0% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 75 is a member of Genetic Group 3.

TR 76 PI 481861

Maras

This accession was collected from Turkey and it was determined to be of an intermediate type with more characteristics of the brasilia variety. It has a slightly ridged stem and ovate leaves that are smooth. When grown in the manner common of flue-cured tobacco, it took an average of 43 days to flower and reached a height of 74.7 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 20.6 cm long and 17.5 cm at its widest point. Its flowers are 23.8 mm long and the limb of the corolla is 15.5 mm in diameter. The capsules, which are rotund in shape, are 1.2 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.0% before plants are topped and 3.3% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 76 is a member of Genetic Group 3.

TR 77 PI 481867

Karabaglar

This accession was collected from Turkey and it was determined to be of the pumila variety. It has a smooth stem and ovate leaves that are slightly puckered. When grown in the manner common of flue-cured tobacco, it took an average of 36 days to flower and reached a height of 71.7 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 21.6 cm long and 18.1 cm at its widest point. Its flowers are 24.8 mm long and the limb of the corolla is 16.6 mm in diameter. The capsules, which are rotund in shape, are 1.2 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.1% before plants are topped and 5.5% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 77 is a member of Genetic Group 3.

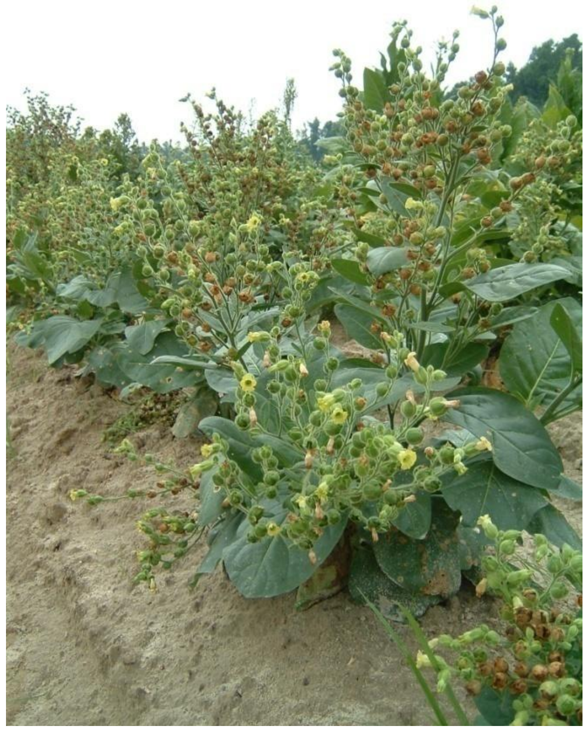
TR 78

*selection from TR36

This accession was selected from within the original seed stock of TR36 because of its different growth habits. This selection tends to be of a more intermediate type than TR36. When grown in the manner common of flue-cured tobacco, it took an average of 32 days to flower and reached a height of 79.5 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 21.8 cm long and 18.3 cm at its widest point. Its flowers are 23.3 mm long and the limb of the corolla is 15.0 mm in diameter. The capsules, which are rotund in shape, are 1.2 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.3% before plants are topped and 4.7% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 78 is a member of Genetic Group 3.

TR 79

*selection from TR42



This accession was selected from within the original seed stock of TR42 because of its different growth habits, though it is still of the pumila variety. It has a smooth stem and broad elliptic or ovate leaves that are smooth. When grown in the manner common of flue-cured tobacco, it took an average of 42.5 days to flower and reached a height of 83.7 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 16.8 cm long and 13.6 cm at its widest point. Its flowers are 22.5 mm long and the limb of the corolla is 14.6 mm in diameter. The capsules, which are rotund in shape, are 1.0 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.8% before plants are topped and 3.7% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 79 is a member of Genetic Group 5.

TR 80

*selection from TR52

This accession was selected from the original seed stock of TR52 because it does not share the thicker dark green leaves of that accession, though it is still of the pumila variety. It has a smooth stem and ovate leaves that are smooth. When grown in the manner common of flue-cured tobacco, it took an average of 36 days to flower and reached a height of 86.2 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 20.5 cm long and 16.8 cm at its widest point. Its flowers are 25.5 mm long and the limb of the corolla is 17.8 mm in diameter. The capsules, which are rotund in shape, are 1.0 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.1% before plants are topped and 3.5% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 80 is a member of Genetic Group 5.

TR 81

This accession was collected from an unknown location and it was determined to be of the pumila variety. It has a smooth stem and broad elliptic or ovate leaves that are smooth. When grown in the manner common of flue-cured tobacco, it took an average of 36 days to flower and reached a height of 64.0 cm, including the inflorescence. A leaf taken from the middle of the plant will be on average 16.0 cm long and 11.8 cm at its widest point. Its flowers are 23.5 mm long and the limb of the corolla is 15.8 mm in diameter. The capsules, which are rotund in shape, are 1.0 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.2% before plants are topped and 5.5% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 81 is a member of Genetic Group 3.

TR 82 PI 555556

This accession was collected from an unknown location and it was determined to be of the brasilia variety. It has a slightly ridged stem and ovate or cordate leaves that are slightly puckered to puckered. When grown in the manner common of flue-cured tobacco, it took an average of 53.5 days to flower and reached a height of 127.0 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 27.1 cm long and 23.5 cm at its widest point. Its flowers are 22.3 mm long and the limb of the corolla is 14.1 mm in diameter. The capsules, which are rotund in shape, are 1.0 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.1% before plants are topped and 5.5% two weeks after topping, but may reach much higher levels when heavily fertilized. TR 82 is a member of Genetic Group 3.

TW 116 PI 243561

N. rustica var. *pavonii*, 44

This accession was collected from the Cajamarca Highlands of Peru and it is documented to be of the pavonii variety. It has a smooth stem and broad elliptic leaves that are smooth. When grown in the manner common of flue-cured tobacco, it took an average of 46.5 days to flower and reached a height of 101.4 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 17.3 cm long and 17.8 cm at its widest point. Its flowers are 23.5 mm long and the limb of the corolla is 14.1 mm in diameter. The capsules, which are elliptical in shape, are 0.8 cm in diameter and generally very small compared to other accessions. The percent total alkaloids is very low— 1.3% before plants are topped and 0.6% two weeks after topping. TW 116 is a member of Genetic Group 2.

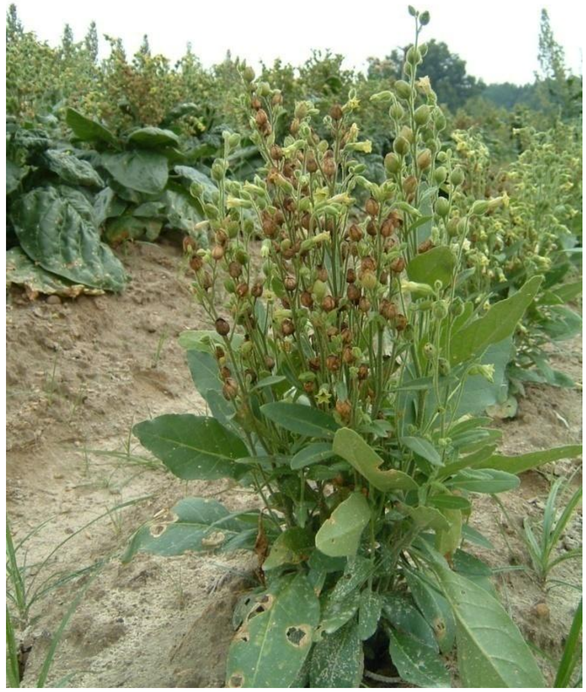
TW 117 PI 555554

N. rustica var. *brasilia*, 48

This accession was collected from an unknown location and it is documented to be of the brasilia variety. It has a slightly ridged stem and ovate or cordate leaves that are puckered. When grown in the manner common of flue-cured tobacco, it took an average of 42.5 days to flower and reached a height of 80.5 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 26.8 cm long and 24.1 cm at its widest point. Its flowers are 22.0 mm long and the limb of the corolla is 13.8 mm in diameter. The capsules, which are rotund in shape, are 0.9 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.1% before plants are topped and 4.4% two weeks after topping, but may reach much higher levels when heavily fertilized. TW 117 is a member of Genetic Group 3.

TW 118 PI 555555

N. rustica var. *pumila*, 49



This accession was collected from an unknown location and it is documented to be of the pumila variety. It has a smooth stem and elliptic leaves that are smooth. When grown in the manner common of flue-cured tobacco, it took an average of 39 days to flower and reached a height of 46.7 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 10.9 cm long and 4.5 cm at its widest point. Its flowers are 21.3 mm long and the limb of the corolla is 12.4 mm in diameter. The capsules, which are elliptical in shape, are very small though they are 0.9 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.3% before plants are topped and 1.7% two weeks after topping, but may reach much higher levels when heavily fertilized. TW 118 is a member of Genetic Group 4.

TW 119 PI 555692

N. rustica, 49A

This accession was collected from an unknown location and it was determined to be of an intermediate type. It has a smooth stem and ovate or cordate leaves that are smooth. When grown in the manner common of flue-cured tobacco, it took an average of 57 days to flower and reached a height of 121.3 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 22.6 cm long and 17.6 cm at its widest point. Its flowers are 23.6 mm long and the limb of the corolla is 13.4 mm in diameter. The capsules, which are rotund in shape, are 1.0 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.0% before plants are topped and 5.0% two weeks after topping, but may reach much higher levels when heavily fertilized. TW 119 is a member of Genetic Group 3.

TW 120 PI 555693

N. rustica, 49B

This accession was collected from an unknown location and it was determined to be of an intermediate type. Based on genetic and morphological data, it is very closely related to TW119. It has a smooth stem and ovate or cordate leaves that are smooth. When grown in the manner common of flue-cured tobacco, it took an average of 64 days to flower and reached a height of 125.2 cm, including the inflorescence. An average leaf taken from the middle of the plant will be 20.8 cm long and 16.3 cm at its widest point. Its flowers are 24.3 mm long and the limb of the corolla is 14.8 mm in diameter. The capsules, which are rotund in shape, are 0.9 cm in diameter. The percent total alkaloids, which is primarily nicotine, is 1.3% before plants are topped and 3.6% two weeks after topping, but may reach much higher levels when heavily fertilized. TW 120 is a member of Genetic Group 3.