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Diversity of *Nicotiana* species

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Abstract. The genus *Nicotiana* is one of the largest in the Solanaceae family and includes more than 80 species. The most well-known and widespread species of the genus *Nicotiana* is tobacco (*Nicotiana tabacum*), within which there are numerous cultivars. Tobacco is one of the most important industrial plants in Poland and worldwide. The great diversity within the genus makes it an excellent source of variation in a narrowing gene pool and can be used in breeding programmes. Studies of *Nicotiana* species also concern mechanisms of polyploidisation and evolution. There are also model species within the genus. However, in order to make full use of the collected germplasm resources, a detailed knowledge of the collection materials is necessary. While there are various reports in the international literature describing specific issues, the aim of this paper is to indicate the diversity of species in the genus *Nicotiana* as a whole on the basis of our own research and available studies. This review covers the characterisation of the genus *Nicotiana* in terms of origin and geographical distribution, as well as cytogenetic and molecular differences between species. An important aspect is the presentation of the morphological diversity of *Nicotiana* accessions and the variation in the most important tobacco alkaloids. A very important issue is the resistance of *Nicotiana* species to bacterial, fungal and viral diseases, which allows their use in resistance breeding.

Keywords: *Nicotiana*, tobacco, morphological diversity, genetic variation, collection

INTRODUCTION

The genus *Nicotiana* is highly diverse in terms of morphology, chromosome number and geographical distribution, and varies in alkaloid composition and disease and pest resistance. The high diversity of species within the genus *Nicotiana* makes them a valuable gene pool for use in breeding work. Simultaneously, breeding and selecting for the best genotypes with specific traits results in a reduction in genetic diversity. The conservation of gene resources is therefore a priority issue. For this reason, it is necessary to establish collections where, under controlled conditions, proper growth and development of *Nicotiana* accessions is possible and the resulting seeds are kept viable and thus available for research and breeding.

Collections of the genus *Nicotiana* are maintained in several centres in Europe and around the world. These include Bergerac Seeds and Breeding, established to replace the former Tobacco Institute in Bergerac, France (www.bergeracsb.com), and two centres in Germany. The first, under the name NiCoTa, is located in Baden-Württemberg and the second is the IPK gene bank in Gatersleben (Lewis, 2021). The US collection of gene resources of the genus *Nicotiana* is located at the Oxford Research Station in Oxford, North Carolina, and in addition, smaller quantities of seeds in the form of duplicates are stored long-term at -20°C at the National Genetic Resource Centre in Fort Collins, Colorado. The largest *Nicotiana* collection is located at the Tobacco Research Institute (TRI) of the Chinese Academy of Agricultural Sciences (CAAS) in Shandong Province (TRI, 2016).

The origins of the gene resources of the genus *Nicotiana* at Puławy date back to the 1920s, when prof. Lucjan Kaznowski began collecting cultivars and wild species as sources of disease resistance and to improve the breeding materials in terms of quality traits. With the development of breeding, there was an increasing need to collect and evaluate new species and cultivars, which were obtained from many scientific institutions around the world and incorporated into the emerging collection (Czubacka, 2022). The collection of the genus *Nicotiana* currently maintained at the Institute of Soil Science and Plant Cultivation – State Research Institute is one of the largest in Europe and includes 1008 accessions. It comprises 145 accessions belonging to the wild *Nicotiana* species, including 64 species and autotetraploid forms and botanical cultivars, as well

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as 780 cultivars of *Nicotiana tabacum* and 83 cultivars of *Nicotiana rustica*. The collection is deposited in the form of seeds in a long-term storage facility at the National Centre of Plant Genetic Resources at the Plant Breeding and Acclimatization Institute in Radzików (Czubacka, 2022), while duplicates are stored in seed cabinets at 4°C at the Institute of Soil Science and Plant Cultivation – State Research Institute in Puławy.

Nicotiana tabacum is the species with the largest number of cultivars and forms in the entire genus *Nicotiana*, and thus the most numerous in gathered collections. It is also one of the most important industrial plants, cultivated in nearly 100 countries around the world and used mainly for the production of tobacco products (Berbeć, Madej, 2012). A second extremely important application of tobacco is its use in so-called molecular agriculture as a green bioreactor for the production of biopharmaceuticals. In this way, antibodies, vaccines, cytokines and biopolymers and enzymes are produced. This is possible because a very efficient genetic transformation of this plant has been developed and because it has a high biomass production potential (Przybyś, 2012).

THE ORIGIN AND SYSTEMATICS OF THE GENUS *NICOTIANA*

The genus *Nicotiana* is the fifth largest genus belonging to the family Solanaceae. In 2020, 82 species were included (Knapp, 2020), but this number is currently higher due to the discovery of new species (Bally et al., 2021; Chase et al., 2021a, 2021b, 2021c, 2021d, 2021e; Chase, Christenhusz, 2021a, 2021b; Augsten et al., 2022; Chase et al., 2022).

The first studies related to the genus *Nicotiana* took place as early as 1753, when Linnaeus described four species: *N. glutinosa*, *N. tabacum*, *N. rustica* and *N. paniculata.* Later, in 1818 Lehman added 21 species and distinguished the genus as a whole. A further 7 species were added by Kurth. The first systematic division was created by Don in 1838 and included four sections distinguished on the basis of flower colour and shape (Knapp et al., 2004). Subsequently, Kostoff (1943) divided the genus *Nicotiana* into eight sections and included 47 species. Based on a morphological and cytological evaluation, Goodspeed (1954) developed a systematics in which he distinguished three subgenera (*Tabacum, Rustica* and *Petunioides*) and 14 sections, which included 60 species. This author considered that the genus *Nicotiana* had two ancestors: *pre-Cestrum* and *pre-Petunia*, with a basic chromosome number of n=6. The emergence of new *Nicotiana* species was related to the process of spontaneous interspecific crossbreeding and the subsequent doubling of the hybrid's chromosome number. This systematics was in place until 2004. Molecular studies of nuclear (Chase et al., 2003) and plastid DNA (Clarkson et al., 2004) made it possible to revise and slightly alter the systematics of the genus *Nicotiana.* These changes concerned the resignation of the division into subgenera and leaving only sections, within which some rearrangements were made (Knapp et al., 2004). The section *Thyrsiflorae* was abolished and the species *N. thyrsiflora* belonging to it was included in the section *Undulatae*. A new section *Sylvestres* was created for the species *N. sylvestris*. Species belonging to the two sections *Repandae* and *Nudicaulis* were assigned together to section *Repandae* due to common ancestors. The name of several sections was changed: *Genuinae* to *Nicotiana*, *Acuminatae* to *Petunioides* and *Bigelovianae* to *Polydicliae*, and two species were renamed: *Nicotiana trigonophylla* to *Nicotiana obtusifolia* and *Nicotiana bigelovii* to *Nicotiana quadrivalvis*. The last change involved moving the species *Nicotiana glutinosa* from section *Tomentosae* to section *Undulatae*, and the species *Nicotiana glauca* from section *Paniculatae* to section *Noctiflorae*. The systematics currently in use is one according to Knapp et al. (2004) (Table 1).

DIFFERENTIATION OF *NICOTIANA* ACCESSIONS IN TERMS OF GEOGRAPHICAL DISTRIBUTION

Species of the genus *Nicotiana* occur naturally in North and South America, Australia and New Zealand, and Africa (Goodspeed, 1954; Chase et al., 2003, Doroszewska et al., 2009). The largest number of species in the genus *Nicotiana*, belonging to as many as 6 sections (*Alatae, Noctiflorae, Paniculatae, Sylvestres, Tomentosae, Undulatae*), are native to South America. Only the species *N. glauca,* from section *Noctiflorae*, is also found in Central America, the USA and Hawaii, as well as in Australia, Africa, Palestine and India. It is even considered an invasive plant in some countries. Because of its industrial use, *N. tabacum* and *N. rustica*, although native to South America, are cultivated worldwide. Species from three sections (*Polydicliae, Repandae, Trigonophyllae*) are found only in the United States and Mexico. In contrast, eight species, belonging to section *Petunioides* occur naturally mainly in South America but also in North America. A large majority of species from section *Suaveolentes* occur in Australia and on Pacific islands (Goodspeed, 1954; Chase et al., 2003, Doroszewska et al., 2009). Only the species *N. africana* has been found in Namibia, Africa (Merxmüller, Butler, 1975).

CYTOGENETIC AND MOLECULAR DIVERSITY OF *NICOTIANA* ACCESSIONS

Within the genus *Nicotiana* there are both diploid and polyploid species. We distinguish seven sections: *Noctiflorae, Paniculatae, Petunioides, Sylvestres, Tomentosae, Trigonophyllae, Undulatae* in which species are diploid (n=12) and four sections: *Nicotiana, Polydicliae, Repandae, Rusticae* in which all species are allotetraploid (n=24). A separate group is also made up of aneuploid species which we can find in the section *Alatae* (n=9; n=10) and the section *Suaveolentes* (n=16–32) (Table 1). This great diversity makes them the basis for many studies to better understand the process of polyploidisation and evolution.

Based on cytogenetic studies, including genomic in situ hybridisation (GISH) and molecular sequence data, potential parental species for some allopolyploids have been established. The earliest potential ancestors of *Nicotiana tabacum* were identified, with *N. sylvestris* representing the maternal genome and *N. tomentosiformis* the paternal genome with some contribution from genes belonging to *N. otophora* (Kenton et al., 1993; Chase et al., 2003). A second species of industrial importance, *Nicotiana rustica,* arose from a cross between *N. paniculata* and *N. undulata* (Lim et al., 2004). Furthermore, it was determined that the maternal parent for *N. arentsii* is *N. undulata* and the paternal parent is *N. wigandioides* (Lim et al., 2004). It was also shown that for species from section *Suaveolentes,* the paternal parent was a member of section *Sylvestres*, and the maternal parent could be a hypothetical diploid species containing alleles from both sections *Petunioides* and *Noctiflorae* (Kelly et al., 2012).

The number of species within the section *Suavolentes* has increased over the years due to the discovery and description of new species (Goodspeed, 1954; Burbidge, 1960; Clarkson, Symon, 1991; Symon, Kenneally, 1994; Symon, 1998; Symon, Lepschi, 2007; Chase et al., 2018a, 2018b, 2018c; Chase, Christenhusz, 2018a, 2018b; Bally et al., 2021; Chase, Christenhusz 2021a, 2021b; Chase et al., 2021a, 2021b, 2021c, 2021e, 2021f; Chase et al., 2022). According to Goodspeed (1954), the large number of species in this section is the result of several polyploidisation events. In contrast, phylogenetic results to date suggest that a single event gave rise to the entire group followed by extensive speciation at the polyploid level (Clarkson et al., 2017).

Molecular studies have greatly expanded knowledge of *Nicotiana* species. The technique of sequencing is of particular importance in understanding genomes. One of the first genomes sequenced within the genus *Nicotiana* was the species *N. benthamiana* (Bombarely et al., 2012; Naim et al., 2012). In the first phase of tobacco genome sequencing, its two putative ancestral species, *N. sylvestris* and *N. tomentosiformis,* were sequenced and assembled individually (Sierro et al., 2013). Subsequently, the genomes of three cultivars of *N. tabacum* belonging to different functional types were sequenced: K326 (flue-cured light tobacco), TN90 (air-cured light tobacco) and Basma Xanthi (oriental tobacco) (Sierro et al., 2014; Edwards et al., 2017). Genome information by sequencing was also obtained for species: *N. otophora* (Sierro et al., 2014), *N. attenuata* and *N. obtusifolia* (Xu et al., 2017), *N. glauca* (Khafizova et al., 2018) as well as *N. rustica*, *N. undulata, N. paniculata* and *N. knightiana* (Sierro et al., 2018).

The results of the sequencing of the chloroplast genome of *N. tabacum* (Shinozaki et al., 1986) as well as ones of *N. sylvestris* and *N. tomentosiformis* (Yukawa et al., 2006) indicate that the degree of identity of the chloroplast genome of *N. tabacum* with *N. sylvestris* is 99.99% and with *N. tomentosiformis* 98.54%. On this basis, it can be confirmed that the source of the chloroplast genome of *N. tabacum* is *N. sylvestris* (Yukawa et al., 2006). The study by Bland et al. (1985) allow us to conclude that also the mitochondrial genome in *N. tabacum* is derived from *N. sylvestris* although in this case there is a slight evolutionary divergence between these genomes.

The *Nicotiana tabacum* genome is one of the largest in the Solanaceae family (Bakaher, 2020). For this reason, the first version of a high-resolution genetic map constructed using approximately 300 microsatellite markers grouped into 24 linkage groups was only published in 2007 (Bindler et al., 2007), followed by a more detailed map containing 2317 microsatellite markers in 2011. Microsatellite markers were assigned to their respective genome of origin, *N. sylvestris* or *N. tomentosiformis* (Bindler et al., 2011). Another genetic map of tobacco was based on single-nucleotide polymorphism (SNP). This map contained 4,138 SNP markers mapped to 24 linkage groups (Xiao et al., 2015). SNP maps significantly improve the assessment of genetic diversity in tobacco, QTL (Quantitative Trait Loci) mapping or association analyses, and allow more genomic regions to be mapped. This allows the identification of candidate genes associated with these regions. As an example, tobacco cultivars in the Burley type have a strong chlorophyll deficiency phenotype conditioned by a double homozygous recessive genotype at the Yellow Burley 1 (YB1) and Yellow Burley 2 (YB2) loci. Mutations at the YB loci have been shown to cause changes in tobacco leaf chemistry in the form of increased levels of alkaloids and leaf nitrate nitrogen (NO_3-N), which may contribute to the higher levels of Tobacco-Specific Nitrosamines (TSNAs) in these plants (Edwards et al., 2017). In a study by Thimmegowda et al. (2018), SNP discovery efforts were undertaken through whole-genome resequencing of 18 genotypes of Flue-Cured Virginia (FCV) tobacco that differed in dried leaf yield, flavour and nicotine content. The aim of discovering SNP variants was to be able to use them to develop SNP markers for tobacco yield and quality traits.

Cheng et al. (2019) identified a large number of SNP markers and constructed a high-density SNP genetic map for tobacco applying restriction site-associated DNA sequencing with the use of the Illumina HiSeq 2000 sequencing platform. The number of 13,273 SNP markers were mapped to 24 high-density genetic linkage groups in tobacco to identify QTL associated with CMV (cucumber mosaic virus) resistance. Seven CMV resistance QTLs belonging to linkage groups 5 and 8 were mapped which will facilitate marker-assisted selection for this resistance in tobacco.

The first published study of QTL in tobacco was focused on agronomic traits, leaf quality, chemical composition and smoke properties (Julio et al., 2006). Two loci related to leaf surface constituents (cis-abienol and sucrose esters) have been identified that contribute to the flavour

Table 1. Systematics of genus *Nicotiana* according to Knapp et al. (2004) with later additions.

	Taxon	Chromosome number (2n)
	1	2
Section: *Alatae*		
▪	*Nicotiana alata* Link et Otto	18
▪	*Nicotiana azambujae* L.B. Smith et Downs	unknown
▪	*Nicotiana bonariensis* Lehmann	18
▪	*Nicotiana forgetiana* Hort ex Hemsley	18
▪	Nicotiana gandarela Augsten & Stehmann	20
▪	*Nicotiana langsdorffii* Weinmann	18
▪	*Nicotiana longiflora* Cavanilles	20
▪	*Nicotiana mutabilis* Stehmann et Samir	18
▪	*Nicotiana plumbaginifolia* Viviani	20
Section: *Nicotiana*		
▪	*Nicotiana tabacum* L.	48
Section: *Noctiflorae*		
▪	*Nicotiana acaulis* Spegazzini	24
▪	*Nicotiana ameghinoi* Spegazzini	unknown
▪	*Nicotiana glauca* Graham	24
▪	*Nicotiana noctiflora* Hooker	24
▪	*Nicotiana paa* Martinez Crovedo	24
▪	*Nicotiana petunioides* (Grisebach) Millan	24
Section: *Paniculatae*		
▪	*Nicotiana benavidesii* Goodspeed	24
▪	*Nicotiana cordifolia* Philippi	24
▪	*Nicotiana cutleri* D'Arcy	24
▪	*Nicotiana knightiana* Goodspeed	24
▪	*Nicotiana paniculata* L.	24
▪	*Nicotiana raimondii* Macbride	24
▪	*Nicotiana solanifolia* Walpers	24
Section: *Petunioides*		
▪	*Nicotiana acuminata* (Graham) Hooker	24
▪	*Nicotiana attenuata* Torrey ex S. Watson	24
▪	*Nicotiana corymbosa* Remy	24
▪	*Nicotiana linearis* Philippi	24
▪	*Nicotiana longibracteata* Philippi	unknown
▪	*Nicotiana miersii* Remy	24
▪	*Nicotiana pauciflora* Remy	24
▪	*Nicotiana spegazzinii* Millan	24
Section: *Polydicliae*		
▪	*Nicotiana clevelandii* Gray	48
▪	*Nicotiana quadrivalvis* Pursh	48
Section: *Repandae*		
▪	*Nicotiana nesophila* Johnston	48
▪	*Nicotiana nudicaulis* Watson	48
▪	*Nicotiana repanda* Willdenow ex Lehmann	48
▪	*Nicotiana stocktonii* Brandegee	48
Section: *Rusticae*		
▪	*Nicotiana rustica* L.	48
Section: *Suaveolentes*		
▪	*Nicotiana africana* Merxmüller et Buttler	46
▪	*Nicotiana amplexicaulis* Burbidge	36
▪	*Nicotiana benthamiana* Domin	38
◊	*Nicotiana bilybara* Chase & Christenhusz	38
▪	*Nicotiana burbidgeae* Symon	42
◊	*Nicotiana candelabra* Chase & Christenhusz	38

Table 1 continuation

1	2
▪ *Nicotiana cavicola* Burbidge	46
▪ *Nicotiana debneyi* Domin	48
▪ *Nicotiana excelsior* (Black) Black	38
▪ *Nicotiana exigua* Wheeler	32
◊ *Nicotiana faucicola* Chase & Christenhusz	unknown
▪ *Nicotiana fragrans* Hooker	48
◊ *Nicotiana gascoynica* Chase & Christenhusz	40
▪ *Nicotiana goodspeedii* Wheeler	40
▪ *Nicotiana gossei* Domin	36
▪ *Nicotiana hesperis* Burbidge	42
▪ *Nicotiana heterantha* Kenneally et Symon	48
◊ *Nicotiana hoskingii* Chase, Palsson & Christenhusz	unknown
▪ *Nicotiana ingulba* Black	40
◊ *Nicotiana insecticida* Chase & Christenhusz	42
◊ *Nicotiana karijini* Chase & Christenhusz	unknown
▪ *Nicotiana maritima* Wheeler	32
▪ *Nicotiana megalosiphon* Heurck et Mueller Arg.	40
◊ *Nicotiana monoschizocarpa* Symon & Lepschi	48
◊ *Nicotiana murchisonica* Chase & Christenhusz	42
◊ *Nicotiana notha* Chase & Christenhusz	64
▪ *Nicotiana occidentalis* Wheeler	42
◊ *Nicotiana paulineana* Newbigin & Waterh.	32
◊ *Nicotiana pila* Chase & Christenhusz	unknown
▪ *Nicotiana rosulata* (S. Moore) Domin	40
▪ *Nicotiana rotundifolia* Lindley	44
◊ *Nicotiana rupestris* Chase & Christenhusz	38
◊ *Nicotiana salina* Chase, Fay & Christenhusz	unknown
◊ *Nicotiana scopulorum* Chase & Christenhusz	unknown
▪ *Nicotiana simulans* Burbidge	40
▪ *Nicotiana stenocarpa* Wheeler	40
▪ *Nicotiana suaveolens* Lehmann	32
▪ *Nicotiana truncata* Symon	unknown
▪ *Nicotiana umbratica* Burbidge	46
▪ *Nicotiana velutina* Wheeler	32
◊ *Nicotiana walpa* Chase, Dodsworth & Christenhusz	40
▪ *Nicotiana wuttkei* Clarkson et Symon	32
◊ *Nicotiana yandinga* Chase & Christenhusz	42
Section: *Sylvestres*	
▪ *Nicotiana sylvestris* Spegazzini et Comes	24
Section: *Tomentosae*	
▪ *Nicotiana kawakamii* Y. Ohashi;	24
▪ *Nicotiana otophora* Grisebach	24
▪ *Nicotiana setchellii* Goodspeed	24
▪ *Nicotiana tomentosa* Ruiz et Pavon	24
▪ *Nicotiana tomentosiformis* Goodspeed	24
Section: *Trigonophyllae*	
▪ *Nicotiana obtusifolia* M. Martens et Galeotti	24
▪ *Nicotiana palmeri* Gray	24
Section: *Undulatae*	
▪ *Nicotiana arentsii* Goodspeed	48
▪ *Nicotiana glutinosa* L.	24
▪ *Nicotiana thyrsiflora* Bitter ex Goodspeed	24
▪ *Nicotiana undulata* Ruiz et Pavon	24
▪ *Nicotiana wigandioides* Koch et Fintelmann	24

◊ species included in the systematics after 2004 (Bally et al., 2021; Chase, Christenhusz, 2021a, 2021b; Chase et al., 2021a, 2021b, 2021c, 2021d, 2021e; Augsten et al., 2022; Chase et al., 2022) and aroma characteristics of oriental tobacco (Vontimitta et al., 2010). *NtCPS2* is the main gene controlling cis-abienol production in tobacco. A study of tobacco embryos showed that polymorphism in this gene is responsible for the presence or absence of cis-abienol and labdenediol in 90% of the cultivars tested (Sallaud et al., 2013). Furthermore, one of the major QTLs for resistance to blank shank, which is caused by the soil-borne pathogen *Phytophthora parasitica* var. *nicotiana,* co-occurs with loci for cis-abienol (Vontimitta, Lewis, 2012). Other loci for resistance to soil-borne diseases such as tobacco blank shank and bacterial wilt have also been discovered (Drake-Stowe et al., 2017).

Species of the genus *Nicotiana* are used as model plants in genetics, molecular biology and biotechnology research. One of these is *N. tabacum*, which is considered a model organism because of its fairly short growth and development period as well as the ease of modification which allows the expression of transgenic proteins to be studied (Gebhardt, 2016). TBY-2 is the source of one of the most widely used plant cell lines. It has been obtained from seedlings of *N. tabacum* L. cv. Bright Yellow 2 which grows rapidly and reproduces 80 to 100 times in 1 week. Preparation of protoplasts from TBY-2 cells has been developed, from which organelles are easily isolated (Nagata et al., 2004). In addition, *Nicotiana tabacum* is a classic model of an allopolyploid species. Knowing the genomic structure of tobacco would provide a more detailed information on genomic evolution in the Solanaceae family.

The second model species is *Nicotiana benthamiana* (Fig. 1p) (Chase et al., 2021d). This species is found in northern Australia and until recently was considered one of the most widespread species in the section *Suaveolentes* (Chase, Christenhusz, 2018c). The first specimen of *N. benthamiana* was collected in 1936 by John Cleland from the Adelaide Institute during an expedition to the north-west coast of Australia (Wylie, Li, 2022). Detailed studies performed recently using phylogenetic and population genetic analyses have revealed the existence of five distinct species showing morphological and geographical differences within the population considered to be *N. benthamiana* (Cauz-Santos et al., 2022). These include *N. bilybara, N. candelabra, N. rupestris, N. scopularum* and the correct *N. benthamiana* (Chase et al., 2022). Within the *N. benthamiana* accessions, an LAB strain has been isolated that has been widely used in plant/virus interaction studies due to its exceptional susceptibility to a wide range of plant viruses. The species is also relatively easy to manipulate *in vitro* and is a good for transgene and recombinant protein expression and for experiments using CRISPRCas9 and other gene editing systems. For this reason, it is also used for the production of pharmaceuticals (Pombo et al., 2020; Chase et al., 2021d; Wylie, Li, 2022). The LAB strain has been found to have a 72-nucleotide insertion mutation in the RNA-dependent RNA polymerase gene (*NbRdr1m* gene), rendering it non-functional and causing viral susceptibility. However, there are wild forms of *N. benthamiana* that contain an intact, functional copy of the *NbRdr1* gene. There are also morphological differences between the wild form of *N. benthamiana* and the LAB strain, whose plants are shorter, have thinner and more flexible stems and petioles, smaller and softer leaves with a lighter shade of green and smaller flowers. Furthermore, the seeds of the LAB form are larger and do not need a dormancy period, making faster reproduction possible (Wylie, Li, 2022). A thorough study of the accession, and the saved correspondence that was carried out between scientific centres, led to the conclusion that the LAB strain originated from Cleland's first collection. The seeds he collected were sent to Thomas Goodspeed who conducted extensive research on the genus *Nicotiana,* and were subsequently sent to other laboratories around the world (Chase et al., 2021d; Wylie, Li, 2022).

The model species used to study ecological interactions occurring in nature is *Nicotiana attenuata*. It is a diploid species found in the United States. The plant has adapted to an ecological niche defined by the post-fire environment, where soils are typically nitrogen-rich and biotic stresses are highly dynamic. *Nicotiana attenuata* exhibits high biochemical and phenotypic plasticity that allows it to cope with environmental challenges associated with pollinators, microbes and insect herbivory. This species has been the subject of detailed genomic, transcriptomic and metabolomic studies (Brokmöller et al., 2017; Navarro-Quezada et al., 2020).

MORPHOLOGICAL DIFFERENTIATION OF *NICOTIANA* ACCESSIONS

Nicotiana species show a great variation in terms of plant habit, inflorescence structure and flower shape and colour as well as leaf shape and size (Doroszewska et al., 2009; Knapp, 2020).

Species in the section *Alatae* are annual plants 0.3 to 1.5 metres high. They form a rosette of leaves during the initial growth phase and next the lower leaves reach a fairly large size while the upper leaves are smaller and lanceolate. The petioles are winged. The inflorescence takes the form of a panicle or false raceme. The flowers open mostly in the evening and are fragrant. In some species they are self-incompatible. The flower colour varies: white (*N. alata* (Fig. 1a), *N. bonariensis),* whitish lavender (*N. longiflora* (Fig. 1b), *N. plumbaginifolia* (Fig. 1c)), greenish yellow (*N. langsdorfii* (Fig. 1d)) and reddish purple (*N. forgetiana* (Fig. 1e)), *N. gandarela*). In contrast, the flowers of the species *N. mutabilis* (Fig. 1f), discovered and described by Stehmann et al. in 2002, have the ability to change colour from white through pink to fuchsia.

The section *Nicotiana* includes only the species *Nicotiana tabacum* (Fig. 1g). It is an annual plant, reaching 1 to 3 metres in height. Within this species, there is a huge number of cultivars showing morphological differences regarding leaf shape and size and flower colour.

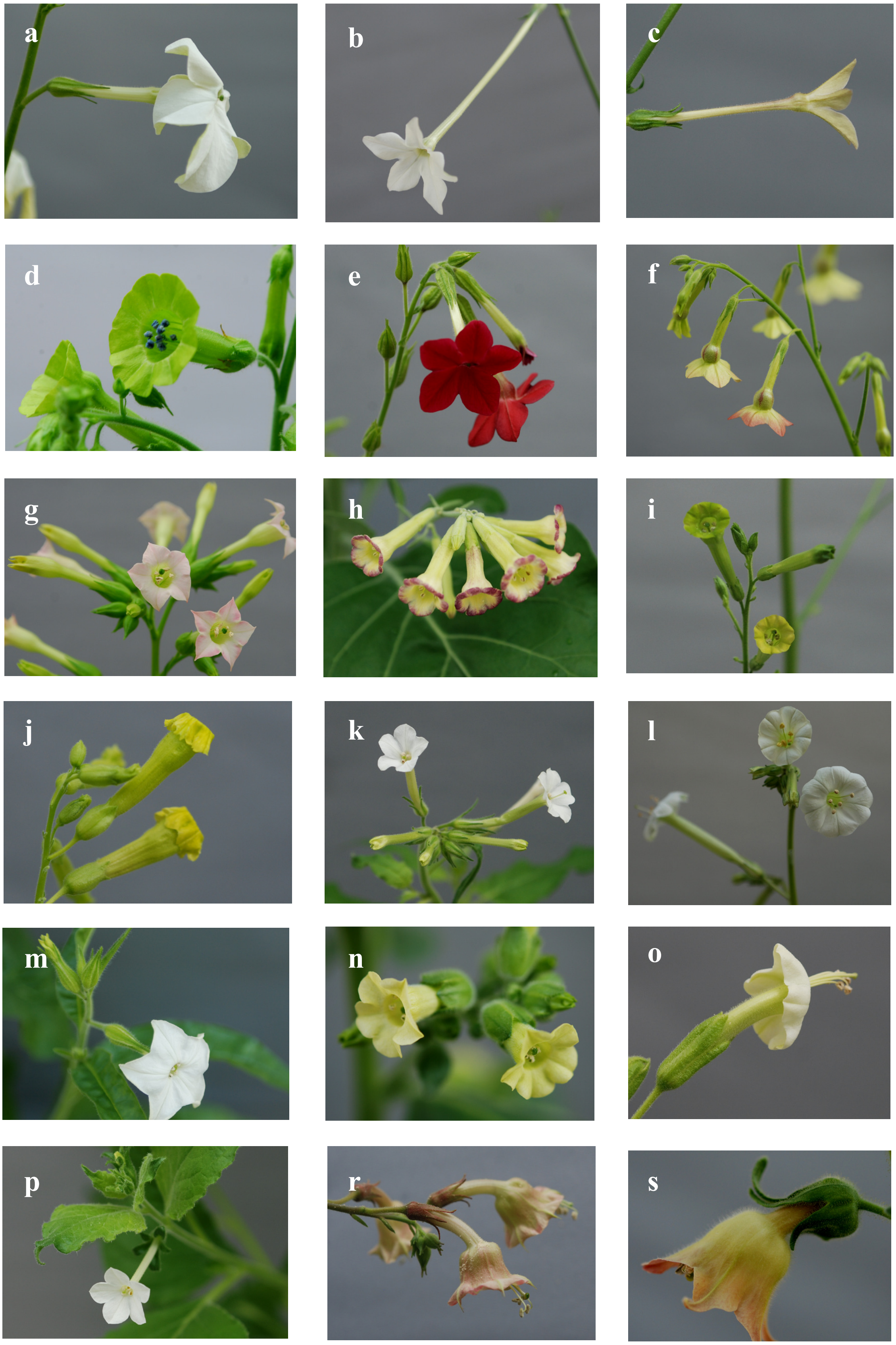

Figure 1. Morphological variation of flowers within the *Nicotiana* collection maintained at the Institute of Soil Science and Plant Cultivation – State Research Institute: a – *N. alata*, b – *N. longiflora*, c – N. *plumbaginifolia*, d – *N. langsdorfii*, e – *N. forgetiana*, f – *N. mutabilis*, g – *N. tabacum*, h – *N. cordifolia*, i – *N. paniculata*, j – *N. solanifolia*, k – *N. acuminata*, l – *N. pauciflora*, m – *N. quadrivalvis*, n – *N. rustica*, o – *N. africana*, p – *N. benthamiana*, r – *N. kawakamii*, s – *N. glutinosa.* Author – A. Depta.

The section *Noctiflorae* contains species with considerable morphological differences among them. *Nicotiana acaulis*, *N. paa* and *N. ameghinoi* are low perennial plants with small, lanceolate leaves and creamy-white or yellowish-white flowers. *Nicotiana noctiflora* and *N. petunioides* are rather annual, self-incompatible species with lanceolate leaves and white flowers that open in the evening. In contrast, *N. glauca,* under natural conditions, is a woody shrub or even a small tree of 3 to 6 metres in height. The stem and leaves are smooth with a greenish-blue colour, and the flowers are yellow.

The *Paniculatae* section includes both annual plants and woody perennial shrubs with rather large, petioled leaves covered with hairs. The inflorescence mainly takes the form of a panicle and the flowers are greenish yellow. This section includes: *N. benavidesii, N. cordifolia* (Fig. 1h), *N. cutleri, N. knightiana, N. paniculata* (Fig. 1i), *N. raimondii, N. solanifolia* (Fig. 1j).

Eight species: *N. acuminata* (Fig. 1k), *N. attenuata, N. corymbosa, N. linearis, N. longibracteata, N. miersii, N. pauciflora* (Fig. 1l), *N. spegazzinii*, belonging to section *Petunioides,* are annuals. The lower leaves form a leaf rosette. The shape and size of the leaves and the type of inflorescence vary. All species in this section have white flowers and the length of the flower tube varies. The flowers of most of them open in the evening.

Nicotiana quadrivalvis (Fig. 1m) (formerly *N. bigelovii*) and *N. clevelandii* are two species belonging to the section *Polydicliae*. They are annual plants with white flowers. The leaves initially take the form of a rosette, while the stem leaves are of various sizes, petioled or petioleless. Both species were cultivated by the Indians as smoking and chewing tobacco.

The *Repandae* section, which includes *N. nesophila, N. nudicaulis, N. repanda* and *N. stocktonii*, comprises small, annual plants with inflorescence in the form of a false raceme or panicle. The lower leaves are quite large, with a winged petiole ending in auricles that clasp the stem or are decurrent. The flowers are white, but the size and length of the corolla tube varies. With the exception of *N. nudicaulis*, the flowers of the other ones open in the evening.

The only representative of section *Rusticae* is the species *Nicotiana rustica* (Fig. 1n). It was the first tobacco species used for smoking, snorting and chewing. Within *N. rustica* there are many cultivars that differ morphologically, but the common feature is that it is an annual plant with rather large, petioled leaves and greenish-yellow flowers.

The *Suaveolentes* section is the most numerous section currently comprising 43 species. The morphological differences within this section concern the height and habit of the plants, the inflorescence and the shape and size of the leaves and flowers. However, a feature common to all species in this group are the white flowers, which in most cases open in the evening and are fragrant. With the exception of *N. africana* (Fig. 1o), *N. fragrans* and *N. gossei*, which can take the form of perennial shrubs, all other species are annual or short-lived herbaceous plants.

A single species of *N. sylvestris*, which belongs to the section *Sylvestres*, is a perennial herbaceous plant reaching a height of 1 to 1.5 metres. Its leaves are large, sessile, with marked auricles, and the flowers are white with a long corolla tube, declineate and mildly fragrant.

The section *Tomentosae* includes five species: *N. kawakamii* (Fig. 1r), *N. otophora, N. setchellii*, *N. tomentosa* and *N. tomentosiformis*, which form woody, sticky shrubs from 1 to more than 5 metres high. The leaves of these species are large, sessile with auricles or winged and decurrent. The flowers take the shape of a calyx, where the corolla tube is yellow-green and the lobes are pink or red.

Two similar species: *N. obtusifolia* (formerly *N. trigonophylla*) and *N. palmeri*, are assigned to the section *Trigonophyllae*. They are annual or less frequently perennial herbaceous plants. They have not very large leaves, often sessile with auriculates clasping the stem. The inflorescence takes the form of a false raceme and the flowers are white with a greenish cream corolla tube, mostly declineate.

The section *Undulatae*, comprising five species: *N. arentsii, N. glutinosa* (Fig. 1s), *N. thyrsiflora, N. undulata* and *N. wigandioides*, is morphologically very diverse in habit, leaf and flower shape and size, and flower colour. In addition, they are both annual plants and perennial shrubs. Despite a number of differences, molecular analysis indicates genetic similarity among these species.

DIFFERENTIATION OF *NICOTIANA* ACCESSIONS IN TERMS OF TOBACCO ALKALOID CONTENT

Tobacco alkaloids are compounds that exhibit physiological effects on human and animals. In low doses they have a stimulating effect on the nervous system but higher doses of alkaloids can have adverse effects on the organism (Trojak-Goluch, Kawka-Lipińska, 2022).

The main alkaloids of species in the genus *Nicotiana* are nicotine and nornicotine, and to a lesser extent anabasine and anatabine (Eich, 2008; Dewey, Xie, 2013). Studies of 64 *Nicotiana* species have shown that for 35 species, including *N. tabacum* and its parent species *N. sylvestris,* nicotine is the dominant secondary metabolite while nornicotine is the dominant alkaloid for 25 species including *N. tomentosiformis* (Sierro et al., 2013). In four species (*N. glauca, N. noctiflora, N. petunioides* and *N. acaulis*), anatabine is the main alkaloid (Sisson, Severson, 1990).

Nicotine takes the form of a colourless, oily liquid that darkens in the air and is characterised by a sharp, long-lasting taste. Nicotine is synthesised in the roots and transported by xylem to the rest of the plant. Nicotine biosynthesis is determined by two genes *NIC1* and *NIC2* (Shoji et al., 2010). Nornicotine is a colourless hygroscopic oily liquid with a slightly pungent odour. Nornicotine is formed from nicotine by a conversion process involving appropriate enzymes. This process is controlled by three genes: *CYP82E4, CYP82E5* and *CYP82E10* (Lewis et al., 2010).

Nicotine content varies and depends mainly on the cultivar and type of use but also on environmental and agrotechnical factors. Low nicotine content (0.3–1%) is found in cultivars belonging to the oriental tobacco. The cultivars within Virginia type are characterised by low to medium nicotine content (1–2.5%.), while Burley and Cuban tobacco cultivars have medium nicotine content (3–4%). The highest nicotine content (4–5%) is in dark tobacco (Mocny Skroniowski type). Environmental factors that affect nicotine content are soil moisture, temperature and sunshine. The nutrient richness of the site is also important, mainly concerning nitrogen and potassium. The agronomic treatments carried out are further elements that regulate the alkaloid content of the plant. This applies both to the planting date and the size of the seedlings. Moreover, the harvesting time has a significant influence on nicotine levels in raw tobacco. The leaves should be harvested when they reach technological maturity (Trojak-Goluch, Kawka-Lipińska, 2022).

The content of nornicotine is variable and dependent on the age of the plant and its floor, but there is also inter-individual variation in populations of cultivars and breeding lines. Plants that convert nicotine into nornicotine are called converters. Plants that contain nicotine as the main alkaloid are called non-converters.

A specific nicotine content in tobacco leaves is a favourable characteristic, as it determines the high quality of the raw material (Trojak-Goluch, Kawka-Lipińska, 2022). In contrast, a high proportion of nornicotine is undesirable due to its ability to be converted into N-nitrozonornicotine (NNN) which belongs to the group of tobacco-specific nitrosamines that have carcinogenic effects (Hecht, 2003).

DIFFERENTIATION OF *NICOTIANA* ACCESSIONS IN TERMS OF RESISTANCE TO PATHOGENS

Nicotiana species possess resistance to a range of diseases and pests (Doroszewska et al., 2009). Unfortunately, in many cases there are great difficulties in transferring resistance genes due to existing barriers to crossability which include cross incompatibility, mortality and infertility of hybrids (Depta et al., 2012). Furthermore, resistance in *Nicotiana* species is often multigene determined. Interspecific crossing is also associated with random introgression of donor genes into the recipient genome, which negatively affects the biological and agronomic traits of the obtained cultivar (Berbeć, Doroszewska, 2020). Nevertheless, wild tobacco species represent a valuable, often the only source of resistance to pathogens, and the development of new techniques makes it possible to overcome the difficulties encountered in the breeding process.

Over many years, breeding work has been carried out to obtain *Nicotiana tabacum* cultivars resistant to the major bacterial, fungal and viral diseases. Within the genus *Nicotiana, Nicotiana longiflora* was the species for which successful attempts were made to transfer resistance to the bacteria *Pseudomonas syringae* and *P. angulata* (Clayton, 1947). In addition, the gene that confers the resistance to nematode (*Meloidogyna javanica*) (Schweppenhauser, 1968) was transferred from *N. longiflora.* Breeding work with *Nicotiana repanda* yielded lines showing partial resistance to *Alternaria alternata* and full resistance to *Cercospora nicotianae* and *Meloidogyna javanica* (Stavely et al., 1973). The transfer of genes conditioning resistance to *Berkeleyomyces basicola* (formerly *Thielaviopsis basicola*) from the species *N. debneyi* has been undertaken by many researchers (Hoffbeck et al, 1965; Clayton, 1969; Miller, 1987; Palakarcheva, 1995; Bai et al., 1996; Brandle et al., 1997). Thus, at the Institute of Soil Science and Plant Cultivation – State Research Institute in Puławy, the flue-cured tobacco cultivars possessing resistance to black root rot were obtained (Berbeć, Trojak-Goluch, 2001). Successful breeding work for resistance to *B. basicola* was also carried out with the use of *N. glauca* (Trojak-Goluch, Berbeć, 2005).

An extremely important issue is obtaining resistance to viral diseases. Within the genus *Nicotiana* there are species resistant to tobacco veinal necrosis caused by potato virus Y (PVY) (Sievert, 1972; Głażewska, 1977; Doroszewska, Depta, 2011). However, breeding work for PVY resistance has been conducted mainly with the use of *N. africana* (Wersman, 1992; Lewis, 2005; Doroszewska 2010). Despite the immunity of *N. africana*, the hybrid forms obtained with *N. tabacum* showed only partial resistance to PVY (Doroszewska, 2007; Lewis, 2007). *Nicotiana alata* is the species showing resistance to a second very important viral pathogen of tobacco – tomato spotted wilt virus (TSWV). Breeding work with this species has been difficult, due to crossability barriers, but the use of various techniques has made possible obtaining hybrid forms and subsequently breeding lines and cultivars resistant to TSWV (Gajos, 1987, 1993; Laskowska, Berbeć, 2010; Trojak-Goluch et al., 2011). Another example concerns tobacco mosaic virus (TMV) resistance which was derived from the species *N. glutinosa* and first transferred by interspecific crossing to the cultivar Samsun (Holmes, 1938).

The above examples do not exhaust the issue of the diversity of resistance in species of the genus *Nicotiana,* but only indicate its richness and possibilities for practical use.

CONCLUSIONS

The genus *Nicotiana* is highly diverse in terms of geographical distribution, chromosome number and morphological characteristics, as well as differing in alkaloid composition and resistance to diseases and pests.

The natural place of origin of wild tobacco species is North and South America, Australia and the Pacific islands and Africa.

Cytogenetic differences within the genus *Nicotiana* have been the basis for creating sections that include single species or collect those that are molecularly similar. However, the genetic similarity of the accessions and the morphological one do not always correspond to each other. In terms of cytology, there are seven sections where species are diploid, four sections with allotetraploids and two sections with aneuploids. Detailed molecular studies revealed that the parental species for *N. tabacum* were *Nicotiana sylvestris* and *Nicotiana tomentosiformis* while for *N. rustica* they were *N. paniculata* and *N. udulata*.

There is great morphological diversity within the genus *Nicotiana*. Most species are annuals, but there are also perennial ones that take the form of shrubs or even small trees. The habit, type of inflorescence, as well as the shape and size of the leaves are specific for species. There are particular differences in the flowers where not only the shape and size but also the colour varies. These characteristics mean that *Nicotiana* species can also be used as ornamental plants.

The main tobacco alkaloids in the genus *Nicotiana* are nicotine and nornicotine as well as anabasine and anatabine but their proportion varies among species. Nicotine is the predominant alkaloid for 35 species, including *N. tabacum* and *N. sylvestris* while nornicotine predominates in 25 species including *N. tomentosiformis*. In contrast, anatabine is the main alkaloid for four species.

Extremely important characteristics of *Nicotiana* accessions are their resistance to a range of pathogens. However, a common problem is the difficulty in transferring resistance due to different crossability barriers. The development of modern biotechnological and molecular techniques makes possible to overcome some of the problems and exploit many desirable sources of variability in practical breeding.

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