Chapter 1
Introductory Notes

1.1 The Importance of Tobacco (*Nicotiana tabacum L.*) and of Other Species in the Genus *Nicotiana*

The genus *Nicotiana* was only recently considered the fifth largest in Solanaceae (Knapp, 2020). Now, owing to a series of unprecedented new discoveries in Australia, it may have acquired the status of the fourth largest. *Nicotiana* constitutes a large and highly diversified assemblage of species ranging from the diminutive root-sprouting *N. acaulis* through annuals and short-lived perennials of different sizes and habits to a small-sized tree (*N. glauca*). The current number of *Nicotiana* species may vary in different publications. At present, the author of this review counted 108 taxa which have been given the specific status or at least treated provisionally as separate species and/or included in some research. Of these, 96 occur (or occurred) in natural habitats, two have been domesticated as commercial crops (*N. tabacum* and *N. rustica*) and one has been synthetized as highly heterogeneous ornamental (*N. sanderae*).

Nicotianae growing in the wild are presently indigenous to many parts of North and South America, Australia and to some offshore islands of those continents. One of those species, or its progenitor, made its way to as far as south-western Africa and became part of the Namibian flora. Most of the *Nicotiana* species are known from their diverse natural habitats such as roadsides, derelict lands, gravelly or rocky riverbeds and ravines, some of them prefer shaded places such as forest margins, shady slopes, rock crevices, cave entrances etc. (Tatemichi, 1990). *Nicotiana glauca* has spread naturally in many parts of the world as a persistent and toxic weed (Bogdanović et al., 2006). Some of the *Nicotiana* species, e. g. *N. obtusifolia*, *N. attenuata*, *N. quadrivalvis*, *N. benthamiana*, *N. gossei*, *N. excelsior*, *N. ingulba* have been transiently domesticated or their leaves were collected from the wild by natives chiefly for ceremonial drugs (Horton, 1981; Tatemichi, 1990). Some others, such as *N. alata* and its hybrids with other members of the section Alatae (*N. forgetiana*, *N. langsdorffii*) and also *N. sylvestris,* have become popular as

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ornamental plants owing to their fragrant or sometimes large and showy flowers. The attraction as garden plants of some short-day *Nicotianae* from the section Tomentosae lies in their decorative purple flowers such as these of *N. setchellii*, but they are also valued because of the sheer height to which they can grow.

For a long time, *N. tabacum* was regarded as almost exclusively a cultivated species whose occurrence was, save for occasional escapes from cultivation, invariably associated with human habitation (Goodspeed, 1954). Since that time an increasing amount of evidence has been presented for tobacco becoming established in many parts of the world and over a range of different habitats as a weed infesting cultivated fields and gardens but also as a naturalized part of the local flora (Randall, 2012; CABI, 2019).

Tobacco (*Nicotiana tabacum* L.) takes a singular place among cultivated plants. It continues to be, along with cotton, one of the two major non-food cash crops worldwide. Another feature of *N. tabacum* is that it is one of two *Nicotianae* whose history of cultivation has reached far beyond its natural range of occurrence. The other is *N. rustica*, known as Aztec tobacco or makhorka. Both *N. rustica* and *N. ta*bacum are the sources of nicotine, but over time *N. tabacum* has outweighed *N. rustica* in importance as a cultivated crop finally to become the sole provider of tobacco leaves as an item of commerce. Cured leaves of tobacco are used in the manufacture of nicotine-containing products mainly in the form of cigarettes but also available in other presentations to be used for smoking, chewing and snuffing. More recently, some of the tobacco crop has been grown for nicotine as the end product to be used as highly diluted aqueous solutions called e-liquids. In a smoking-simulating device called e-cigarette the liquid is atomized into an aerosol that is inhaled by the user. The liquid, apart from nicotine, also contains other additives, some of them potentially carcinogenic, but e-cigarettes are now regarded as a less harmful option to the traditional smoking materials.

As an industrial crop and the source of widely used nicotine-based stimulants tobacco still provides livelihood for rural people in many parts of the world although its importance in many developed countries has been on the decline due to public health-motivated governmental pressures and economic constraints. Tobacco is also unique in that it has become a model plant in both fundamental and applied genetic studies. As a crop plant of substantial economic importance, *N. tabacum* has been subject to regular, conscious and research-based breeding effort that has lasted for more than a century. The latter aspect benefited to a considerable extent from the fact that numerous wild species of the genus *Nicotiana* provide a vast reservoir of potentially usable germplasm for their cultivated relative.

1.2 Hybridization within the Genus *Nicotiana*

The interest in hybrids of *Nicotiana* started in the eighteenth century, long before the breeding of tobacco advanced from lore to science. The major driving motive that made early *Nicotiana* investigators produce and explore the products of different interspecific crosses was concerned with *Nicotiana* phylesis and systematics. At that time, the study of affinities between various *Nicotiana* species drew to a large extent on the behaviour of meiotic chromosomes and on other aspects of cytogenetics in interspecific hybrids. Up to the early 70s of the last century the vast majority of hybrids in *Nicotiana* were probably created with that sole purpose in mind. The interest of geneticists in interspecific hybrids waned considerably with the advent of more sophisticated molecular methods.

Although scaled down, the research on interspecific hybridization in *Nicotiana* has not been abandoned altogether and the wild relatives of tobacco have continued to feature on the breeding agenda of many tobacco laboratories. A large number of *Nicotiana* species have been found to be resistant to common and destructive diseases of the tobacco crop, a valuable asset especially if no resistance could be available within the cultivated species. For the tobacco breeder, cytoplasmic male sterility (CMS) is yet another important benefit that could be accessed through interspecific hybridization. CMS is a prerequisite for technically feasible and economically viable seed production of hybrid cultivars. It looks like the wild *Nicotianae* are practically the sole providers of that important trait for tobacco.

Over more than a century, numerous attempts to hybridize one species of *Nicotiana* with another have resulted in an increasing number of different hybrid combinations and in the refinement of the methods by which those hybrids are obtained. The development of tissue and cell culture techniques and, more recently, also genetic engineering technologies allowed many of the hybridization barriers to be removed or circumvented thereby greatly enlarging the number of species the genomes of which could be combined in interspecific hybrids thus creating opportunities for increased biodiversity from which both nature and man could benefit.

1.3 Types of Interspecific Hybrids in *Nicotiana*

In this review, three processes are discussed by which the genome of one species can become united with the genome of another and thus form an interspecific hybrid. Two of these processes are well documented and generally accepted, the third was and, to some extent, still is the subject of major controversy:

(a) **sexual hybrids** that arise from union of two gametes, female and male, which is done by fertilizing the egg cells of the female parent by the pollen of the male parent. The process occurs in nature and is also imitated in experimental work. In the latter case, various modifications, unknown in nature, have been introduced to facilitate fertilization and, in many cases, to make it feasible.

(b) **somatic hybrids** that arise through the union of naked somatic protoplasts from different species that are isolated and induced to fuse into one cell (protoplast fusion). By dividing and organ differentiation, a process analogous to that occurring in sexual reproduction, the fused cell gives rise to a hybrid plant. Protoplast fusion bypasses the sexual reproductive path and generally requires a sophisticated laboratory and refined methods to be accomplished. Until very recently, the basic mechanism of somatic hybridization was thought to be entirely alien to nature. Recent discoveries, however, seem to have thrown new light on the significance of what is called horizontal transfer in natural evolutionary processes and possibly created a new method by which interspecific hybrids can be produced.

(c) **graft hybrids** that arise through the fusion of two plants or their parts called stock and scion and which were purported to be a source of novel hereditary variation. Historically, graft hybrids were advocated in some communist-controlled countries as a breakthrough in plant genetics and breeding that had invalidated the principles of 'mendelism-morganism'. The tenets of the "revolutionary" biology and genetics, saturated with ideological apriorisms and ill-supported by scientific evidence, were generally treated with extreme skepticism by the majority of conventional geneticists of the time. The concept of graft hybrids, as it was understood in the 1940s and 1950s, was ultimately abandoned altogether but some of its aspects have been given a new life by recent discoveries that involved *Nicotiana*

1.4 Terminology and Usage in Relation to Interspecific Hybrids and Introgression in This Book

(a) hybrid e.g. *N. tabacum* × *N. glutinosa*, unless otherwise specified, usually means an amphihaploid hybrid from mating female parent *Nicotiana tabacum* with male (pollen) parent *Nicotiana glutinosa*; such an example hybrid contains a single (haploid) genome of *N. tabacum* (T $n = 24$) and a single (haploid) genome of *N. glutinosa* (G $n = 12$) and is an amphihaploid (TG). If the amphihaploid status of the hybrid is to be emphasized the notation 2x (*N. tabacum* x *N. glutinosa*) is used; Alternatively, F_1 (*N. tabacum* x *N. glutinosa*) or, simply, *N. tabacum* x *N. glutinosa* denotes the first generation from mating the two species; in either case, name/designation of the maternal species/genome comes first, unless indicated otherwise
Note: In this review, while due recognition is given to the allopolyploid origin of *N. tabacum* and several other *Nicotiana* species, they are treated as functional diploids, thereby avoiding semantic confusion when experimentally created hybrids of different ploidy levels are discussed.

(b) reciprocal hybrid: hybrid with the reversed order of the maternal and paternal species e.g., *N. glutinosa* × *N. tabacum* is the reciprocal of *N. tabacum* × *N. glutinosa*.

(c) amphidiploid means a hybrid in which two genomes of each parental species are combined, e.g.: 4x (*N. tabacum* × *N. glutinosa*), (TTGG).

(d) consequently, sesquidiploid contains a doubled chromosome complement of one parental species and a single complement of the other species, even though the author is aware that that, e.g., the sesquidiploid 3x (*N. tabacum* ×

N. glutinosa) and many others discussed in this review are phylogenetic allopentaploids. Thus (SylSylTomTomGlu) is composed of two ancestral doubled, tomentosoid and sylvestroid, subgenomes (SylSylTomTom) contributed by *N. tabacum* and a single genome contributed by *N. glutinosa* (Glu).

(e) allopolyploid signifies any hybrid that contains multiplications of the basic chromosome complements of distinct species without specifying the number of constituent genomes and species.

(f) aneuploidy refers to a deficiency or an excess of one or more chromosomes *vis-à-vis* normal or expected chromosome complement.

(g) polysomy/nullisomy refers to deficiency or multiplication for a particular chromosome in diploid genomes where normally all chromosomes are present in duplicate. Thus, nullisomic means deficient for the whole pair of homologous chromosomes, monosomic refers to the presence of only one chromosome of a particular chromosome pair; disomic means the normal diploid condition for a particular chromosome pair; trisomic, tetrasomic etc. denote respective extra multiplications of a particular chromosome in the genome.

(h) chromosome pairing: (in gametogenesis) association of structurally similar or identical chromosomes in the first reductional division also referred to as conjunction or, less frequently, as conjugation.

(i) "Drosera scheme" chromosome pairing – chromosome pairing characteristic of hybrids in which the number of paired chromosomes is the same as the haploid number of chromosomes of the parental species with the lower chromosome number (Goodspeed, 1945, 1954). It is found in interspecific hybrids between amphidiploids and one of their diploid progenitor species. Similar pairing behavior may be also shown by hybrids between diploids and autotriploids of the same species.

(j) trivalent, bivalent, univalent – associations of three, two or of a single chromosome left without a pair during the first reductional division, respectively.

(k) homology, homeology – homology refers to structural identity of chromosomes within the genome of the same species; homeology implies structural similarity of chromosomes belonging to different species.

(l) homoploidy, heteroploidy – refers to evolutionary events that involve hybridization as part of the speciation process: homoploid origin refers to hybridization that is not followed by the change in chromosome number; heteroploidy refers to multiplication of chromosome number in the speciation process.

(m) alleles – different variants of a gene; diploid organisms may be homozygous (two identical alleles), heterozygous (two different alleles) or hemizygous (only a single allele) for a given locus.

(n) alloplasmic – having a nuclear genome combined with a cytoplasm of another species.

(o) CMS, cms – cytoplasmic male sterility or related to cytoplasmic male sterility.

(p) manifestations of cytoplasmic male sterility:

   (i) staminal male sterility – refers to either total absence of male organs (stamens) or their different degrees of degeneration: carpeloid stamens that resemble female organs or their parts (e.g. stigmatoid anthers are anthers transformed into stigma-like structures); petaloid stamens refers to stamens transformed into petal-like structures, and feathery anthers means feather-like degenerative changes of anthers; staminal male sterility is usually pre-meiotic i.e. involves total suppression of male gametogenesis; staminal male sterility is also referred to as structural male sterility

(ii) post-meiotic male sterility – usually is related to normal or nearly normal flower morphology including morphologically normal female organs and normal or nearly normally developed stamens. In post meiotic male sterility the microgametogenesis collapses at various stages and apparently normal anthers are either void of pollen or contain aborted or morphologically normal pollen grains with disabled functionality.

Staminal and post-meiotic male sterility are also referred to as structural and sporogeneous sterility, respectively (Kaul, 1988), although sporogeneous male sterility is a broader concept because it also includes premeiotic stages.

(q) introgression – gene flow from species to species. Natural introgression is an important factor in plant evolution, experimental or applied inrogression is one of the tools used in genetic improvement of plants.

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