

Pollination following grafting introduces efficiently *Ocimum basilicum* L. genes into *Nicotiana tabacum* L.

K. Q. Wei^{1*}, J. X. Yang^{2,3} and Z. Z. Wei³

¹ School of Life Science, Shanxi University, Taiyuan 030006, PR China

² School of Economics and Business Administration, Shanxi University, Taiyuan 030006, PR China

³ College of Agriculture, Shanxi Agricultural University, Taigu 030801, PR China

Abstract

Tobacco is an important cash crop in the world. However, the genetic basis is comparatively narrow among the modern *Nicotiana tabacum* cultivars, limiting its potential for quality improvement. To introduce genes conferring desirable chemical constituents from medicinal plants, a distant hybridization test was conducted between *N. tabacum* and *Ocimum basilicum* L. Seedlings of wild type *Nicotiana sylvestris* and *N. tabacum* cultivar 78-04 respectively acted as rootstock and scion. During the flowering season, hand pollination between 78-04 as pistillate parent and *O. basilicum* as pollen parent was carried out under 22-25°C temperature and 70-80% of relative humidity in the greenhouse. Seed sets of 55% were obtained in 78-04, and about 400 seeds per capsule were produced. But both non-grafted and self-grafted 78-04 plants rarely resulted in fruits by hand pollination and those obtained were without seed. Similar results were obtained in different material combination. The interfamilial F₁ hybrids acquired showed distinct variation with various morphological characteristics, and their hybrid nature was confirmed by isozyme and random amplified polymorphic DNA (RAPD) analyses. This result indicated that pollination following grafting can facilitate gene exchange and recombination at the interfamilial level and efficiently overcome barriers of sexual incompatibility between *N. tabacum* and *O. basilicum*. Our research not only extends the genetic basis of tobacco but also will provide valuable germplasm for improvement of varieties.

Additional key words: basil; tobacco; gene exchanging; pollination; germplasm.

Introduction

The genus *Ocimum* (Lamiaceae) comprises 30-160 annual and perennial herbs and shrubs native to the tropical and subtropical regions of Asia, Africa, and Central and South America (Carović-Stanko *et al.*, 2010). Among all the species *O. basilicum*, known as common basil, has the most economic importance and is cultivated commercially in many countries (Marotti *et al.*, 1996). The leaves of this plant are oval with a sharp tip and the flowers are yellow, white and pink. Traditionally, aromatic leaves, flowering tops and extracted essential oils have been extensively utilized in food as flavoring agents, and in perfumery and traditional medicine. In particular, essential oil has attracted a great deal of interest due to its potential as a sour-

ce of biologically active compounds (Hussain *et al.*, 2008). *O. basilicum* is used in the pharmaceutical industries for its representative chemical quality, such as the spasmolytic, carminative, hepatoprotective, diuretic and stimulating properties (Tsai *et al.*, 2011). Therapeutic effects of *O. basilicum* on respiratory diseases have been reported in the Far East, especially in China and India.

Tobacco (*Nicotiana* spp.) is one of the most important cash crops in the world. It is also widely used in plant breeding and genetics research. The genus *Nicotiana* belongs to family *Solanaceae*, and is classified into three subgenera (*Rustica*, *Tabacum* and *Petunioides*), 14 sections and 60 species. But only two natural amphidiploid species, *Nicotiana tabacum* L. and *Nicotiana rustica* L. with 2n = 48 chromosomes are cultivated for use as tobacco. The former is the

* Corresponding author: keqiangwei1970@gmail.com; kqwei@sxu.edu.cn

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Abbreviations used: PAGE (polyacrylamide gel electrophoresis); RAPD (random amplified polymorphic DNA).

commerce used tobacco in most parts of the world, while the latter is grown extensively in parts of Eastern Europe and Asia Minor. Numerous types of tobacco are defined by different criteria such as method of curing (flue-, air-, sun- and fire-cured tobacco) and morphological and biochemical characteristics (*i.e.*, aromatic fire-cured, bright leaf tobacco, Burley tobacco, Turkish or oriental tobacco) (Gholizadeh *et al.*, 2012). Tobacco cultivars are thought to have arisen from interspecific hybridization between *Nicotiana sylvestris* ($2n = 24$, subgenus *Petunioides*) and *Nicotiana tomentosiformis* ($2n = 24$, subgenus *Tabacum*). Some studies have shown that there is a narrow genetic background in *Nicotiana* species and a high genetic similarity between cultivated tobacco, limiting its potential for improving the quality (Lewis & Nicholson, 2007). In order to broaden the genetic base, new gene pools need to be incorporated into tobacco varieties. Since the chemical constituents of tobacco are very important to the cigarette industry, tobacco breeders become more interested in developing tobacco cultivars possessing the distinctive aroma and potentially reduced harmful characteristics. Cui *et al.* (2007) and Li *et al.* (2008) have tried to transfer chemical components from medicinal plants.

To develop varieties with wide adaptability, higher yield potential and suitable chemical constituents for cigarette industry, plant breeding techniques have been applied to *N. tabacum* for approximately seven decades. However, the genetically engineered cultivars are not currently accepted by the industry and the public

(Moon & Nicholson, 2007). Generally, crossing barriers occur due to the result of incompatibility and incongruity, although wide hybridization has great potential for transferring desirable genes to crops. Up to now, very few instances have been successful in interfamilial hybridization by conventional sexual crosses except protoplast fusion (Kisaka *et al.*, 1997). In this paper, a novel method (*viz.* pollination following grafting) could effectively overcome the interfamilial incompatibility between *N. tabacum* and *O. basilicum*. The F_1 hybrids obtained were confirmed by morphology, isozyme and random amplified polymorphic DNA (RAPD) analyses. This method is being applied in our laboratory to develop new types of tobacco containing active ingredient of *O. basilicum*. The new characteristics would be valuable germplasm for improving tobacco varieties.

Material and methods

Plant materials and study site

O. basilicum ($2n = 48$) seeds used in this study were provided by Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences. The flowers are light pink and the leaves are light green (Fig. 1a). The experiment was conducted on a set of diverse types of accessions (flue-cured and sun-cured): one of *N. sylvestris* (wild *Nicotiana* species), two of *N. rustica* and seven of *N. tabacum* (Table 1). Seeds were obtained

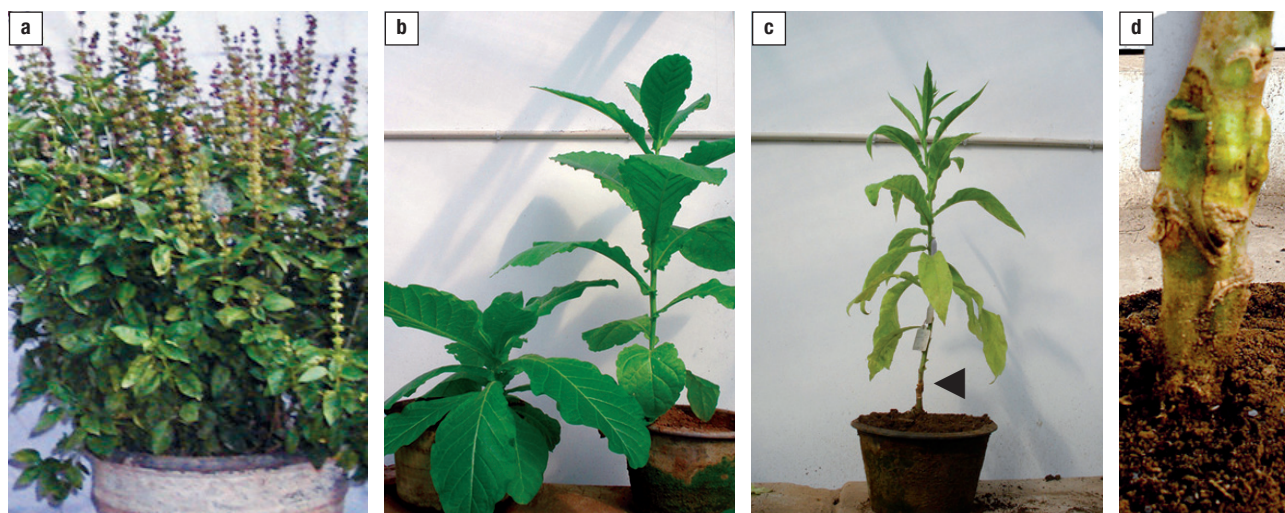


Figure 1. Plant morphological characters of parents. (a): *Ocimum basilicum* L. (b): *Nicotiana sylvestris* (left) and *Nicotiana tabacum* cv. 78-04 (right). (c-d): Graft between *N. sylvestris* (rootstock) and 78-04 (scion). (c): The arrow indicates the node. (d): The healing of the joint.

Table 1. *Nicotiana* germplasm included in this study

Abbreviation	Accession	Species	Variety	Type	Origin
	1273	<i>N. sylvestris</i>	—	Wild	The Andes, from Bolivia to Argentina
HYXY	3518	<i>N. rustica</i>	Hunyuanxiaoye	Landrace	China (Hunyuan, Shanxi)
KLXY	2215	<i>N. rustica</i>	Kelanxiaoye	Landrace	China (Kelan, Shanxi)
	2251	<i>N. tabacum</i>	78-04	Hybrid	China (Taigu, Shanxi)
LY 2	2130	<i>N. tabacum</i>	Longyan 2	Hybrid	China (Mudanjiang, Heilongjiang)
HHY	0173	<i>N. tabacum</i>	Honghuayan	Landrace	China (Wuyang, Henan)
JYH	0520	<i>N. tabacum</i>	Jingyehuang	Landrace	China (Xuchang, Henan)
BXLYJ	1550	<i>N. tabacum</i>	Binxianliuyejian	Landrace	China (Binxian, Heilongjiang)
HLH	0974	<i>N. tabacum</i>	Heilaohu	Landrace	China (Zichang, Shaanxi)
ZED	0638	<i>N. tabacum</i>	Zhuerduo	Landrace	China (Linyi, Shandong)

ned from the National Bank of Tobacco Germplasm Resources in the Tobacco Research Institute, Chinese Academy of Agricultural Sciences. This study was conducted in Taigu County, Shanxi Province which is geographically located in northern China. *N. sylvestris* ($2n = 24$) is the maternal progenitor of cultivated allotetraploid tobacco. The flue-cured tobacco variety 78-04 ($2n = 48$) was developed by the Laboratory of Tobacco Breeding, College of Agriculture, Shanxi Agricultural University from the interspecific hybridization between a Chinese domestic variety 'Jintai 18' and wild species *N. glutinosa* (Kang & Wei, 1986). 'Longyan 2' ($2n = 48$) is bred from 'Mulinghuboxiang' and 'Mu sun-cured tobacco 93-4-5'. 'Hunyuanxiaoye' and 'Kelanxiaoye' ($2n = 48$) are the commonly cultivated *N. rustica* landraces in Shanxi, China, with high adaptability to adverse climatic conditions. Several landraces ($2n = 48$), including 'Heilaohu', 'Honghuayan', 'Zhuerduo', 'Binxianliuyejian' and 'Jingyehuang', were selected to represent China sun- and fire-cured tobacco cultivars with strong disease resistance.

Grafting method

Ten different genotypes of tobacco were seeded in cell flats (cell size $3 \times 3 \times 10$ cm³) filled with peat-lite mixture. According to different combinations, seedlings were grafted at 21 d after sowing by needle graft following the procedure described by Yasinok *et al.* (2009). And non-grafted and self-grafted plants were used as control. After 25 d, plants were transferred to a cultivation chamber under controlled experimental conditions with relative humidity of 60-80%, 25/15°C temperature (day/night) and 16/8 h photoperiod. The plants were grown in pots (50 cm upper diameter,

24 cm lower diameter, 40 cm in height), with one pot containing one plant (Fig. 1b,c,d).

Pollination method

During the flowering season, the hybridization between parental genotypes was done under controlled greenhouse conditions by pollinating the flowers of *N. tabacum* (scion) with the pollen of *O. basilicum*. Fresh *O. basilicum* pollen from just dehisced anthers was collected on a soft brush in the morning, and directly dusted on the stigmas of the emasculated tobacco flowers. The process was repeated 2 or 3 times. These hand-pollinated flowers were bagged to prevent other unwanted pollination and labeled individually with tags until harvest. The number of pollinated flowers, capsules with seeds, seeds produced and 1,000-seed weight was recorded. Seeds harvested from the mature capsules were sowed the following year and grown in the greenhouse. Putative hybrid plantlets were referred to as F₁ plants, and their phenotypes were compared with those of their parents and the hybrid nature was verified by isozyme and RAPD analyses.

Isozyme analysis

Fresh leaves of the parents and five putative F₁ hybrid plants derived from the cross combination [*N. sylvestris* + 78-04] × *O. basilicum* were sampled on 20 d after sowing. Plant materials were ground in 0.1 M Tris-HCl (pH 8.3) using a ratio of 1:2 (fresh weight of leaf to volume of buffer) and were centrifuged at 12,000 rpm for 10 min. The supernatant was electrophoresed with polyacrylamide gel electrophoresis (PAGE) in 3% spacer gel-10% separating gel. For

esterase analysis, the gel was stained with 90 mL phosphate buffer (pH 6.4) containing 90 mg fast blue B salt, 6 mL 2% alpha-naphthyl acetate, 3 mL 2% beta-naphthyl acetate (both dissolved in 1 mL acetone and diluted to 100 mL with 80% ethanol). For peroxidase isozyme analysis, the gel was stained with 100 mL staining buffer containing 20 mL 2% benzidine, 70.4 mg vitamin C, 20 mL 0.6% H₂O₂ and 60 mL distilled water (Xu *et al.*, 2003).

RAPD analysis

When the seedlings developed at least three pairs of true leaves, genomic DNA was extracted from fresh leaves using the cetyltrimethyl ammonium bromide (CTAB) method. Twenty decamer oligonucleotide primers (Sangon Bioengineering Technology Service Co. Ltd., Shanghai, China) (Table 2) were used to amplify genomic DNA of 78-04, *O. basilicum* and five F₁ individuals following the previous protocol (Zhang HY *et al.*, 2008) with small modifications. Amplification reactions were carried out in 25 µL solution containing 2.5 µL 10 × buffer; 2 µL, 2.5 mM dNTPs mixture; 1 µL, 10.0 mM primer; 1 µL genomic DNA; 0.5 µL (2 unit µL⁻¹) Taq polymerase (Roche Diagnostics, Penzberg, Germany). The final volume was made up with HPLC purified distilled water. The Perkin-Elmer Model 480 Thermal Cycler (Perkin-Elmer, Norwalk, CT, USA) was programmed as follows: 94°C 1 min; 37°C 1 min, 72°C 2 min, 94°C 4 min, 40 cycles; 72°C 5 min. Amplified DNA products were electrophoresed on 1.4%

Table 2. Primers used for RAPD analysis

Primer	Primer sequence (5' to 3')	Primer	Primer sequence (5' to 3')
S6	TGCTCTGCCC	S90	AGGGCCGTCT
S11	GTAGACCCGT	S94	GGATGAGACC
S18	CCACAGCAGT	S99	GTCAGGGCAA
S28	GTGACGTAGG	S157	CTACTGCCGT
S36	AGCCAGCGAA	S249	CCACATCGGT
S40	GTTGCGATCC	S265	GGCGGATAAG
S61	TTCGAGCCAG	S290	CAAACGTGGG
S66	GAACGGACTC	S383	CCAGCAGCTT
S68	TGGACCGGTG	S432	CACAGACACC
S73	AAGCCTCGTC	S439	GTCCGTACTG

agarose gels with 0.5 × TBE buffer, stained in ethidium bromide and photographed under UV illumination. These RAPD experiments were repeated at least 3 times and only the repeatable bands were recorded.

Results

Crossability between *N. tabacum* and *O. basilicum*

Large plump capsules were obtained from open-pollinated flowers of the non-grafted and self-grafted plants. Hand pollination of non-grafted and self-grafted tobacco rarely resulted in fruits and those obtained were without seed. But grafting followed by pollination produced up to 426 normal seeds per capsule (Table 3). The cross combination [(*N. sylvestris*

Table 3. Hybrid seed production among the representative cross combinations^a

Materials ^b	Method of pollination	No. of capsules obtained	No. of seeds obtained/capsule ^c	1,000-seed weight (mg) ^c
<i>O. basilicum</i>	Self-pollination ^d		3.9 ± 0.7	3,445 ± 5.1
78-04			1,371 ± 45.2	83 ± 3.3
78-04+78-04			1,364 ± 38.5	83 ± 2.7
<i>N. sylvestris</i> + 78-04			1,330 ± 20.3	89 ± 2.4
78-04 × <i>O. basilicum</i>	Hand-pollination	0	0	0
(78-04 + 78-04) × <i>O. basilicum</i>		0	0	0
(<i>N. sylvestris</i> + 78-04) × <i>O. basilicum</i>		22	426 ± 10.7	67 ± 2.8
(HYXY + LY 2) × <i>O. basilicum</i>		11	197 ± 8.2	52 ± 1.1
(KLXY + JYH) × <i>O. basilicum</i>		0	0	0
(HLH + HHY) × <i>O. basilicum</i>		15	298 ± 8.5	54 ± 2.3
(ZED + BXLYJ) × <i>O. basilicum</i>		3	184 ± 7.1	43 ± 0.9

^a Forty flowers of each material were pollinated artificially at 7-9 am. The temperature was 22-25°C and relative humidity was 70-80% in the greenhouse. ^b A + B indicates rootstock + scion. ^c Each value represents the mean ± SD of the capsules obtained by hand-pollination. ^d A statistical analysis was made among twenty capsules obtained by self-pollination.

Table 4. Effect of temperature and relative humidity on the crossability between *N. tabacum* L. and *Ocimum basilicum* L.^a

Temperature (°C)	Relative humidity (%)	No. of capsules obtained	No. of seeds obtained/capsule ^b	1,000-seed weight (mg) ^b
14-17	60-70	6	382 ± 4.8	61 ± 3.5
14-17	70-80	11	385 ± 4.2	62 ± 3.7
22-25	60-70	4	274 ± 3.5	65 ± 2.4
22-25	70-80	22	426 ± 10.7	67 ± 2.8
22-25	80-90	9	343 ± 5.6	60 ± 3.5
30-33	70-80	5	272 ± 3.4	58 ± 3.0
30-33	80-90	2	169 ± 1.4	49 ± 1.4

^a Seedlings of wild type *Nicotiana sylvestris* and *Nicotiana tabacum* cv. 78-04 acted as rootstock and scion, respectively. During the flowering season, hand pollination between 78-04 as pistillate parent and *Ocimum basilicum* L. as pollen parent was carried out. Under different temperature and relative humidity, 40 flowers were pollinated at 7-9 am in the greenhouse. ^b Each value represents the mean ± SD of the capsules obtained.

rootstock + 78-04 scion) × *O. basilicum*] gave the highest setting percentage (55%) compared with other combinations. Meanwhile, the crossability between *N. tabacum* and *O. basilicum* was markedly affected by temperature and humidity in the greenhouse. Pollination at low temperature (22-25°C) and high relative humidity (70-80%) contributed to the successful probability of hybridization (Table 4). Similar results were obtained in different material combination.

Morphology characteristic of F₁ hybrid

Among different cross combinations [(*N. sylvestris* + 78-04) × *O. basilicum*] was outstanding for hybrid seed sets and seed yield per capsule (about 400). One hundred seeds were planted the next year in the greenhouse, and putative hybrid plants generated obvious phenotypic variation, as observed at flowering time. In general, the morphological traits of the F₁ hybrids looked like *N. tabacum*. Figs. 2a-l present such agronomic performance as leaves, inflorescences and flowers, etc., which could be caused by the introduced DNA from *O. basilicum*. Most importantly, they were apparently completely fertile. Eight individual plants resembled *O. basilicum*, especially in the foliar morphology. Twenty four hybrid plants developed to a height approximately equal to that of 78-04, but distinct changes appeared as the light-colored flowers, leaves shape and the big capsules (Figs. 3a-j). This will facilitate mass selection for desired plant types and leaf characteristics from segregating hybrid populations.

Isozyme analysis

As shown in Figs. 4a,b, analyses with esterases and peroxidases were able to distinguish between *N. tabacum* and *O. basilicum* hybrids and parents and confirm the hybridity of five F₁ plants. The characteristic bands of both parents were not simply represented in the hybrids. Probably due to combination among enzyme subunits or differential gene expression in a different genetic background, the F₁ hybrids showed new bands.

RAPD analysis

Out of 20 RAPD primers assessed, two primers (S28 and S265) gave reproducible results and showed different band patterns specific to *N. tabacum* and *O. basilicum*. These major paternal bands were also found to be present in the five hybrid seedlings (Figs. 5a,b). The RAPD profiles from the two primers gave evidence for hybrid nuclear genome.

Discussion

Owing to the cross-incompatibilities, genetic introgression between distantly related species is very difficult via conventional ways. The graft is an effective agricultural approach to improve the stress resistance and quality of crops (Ruiz *et al.*, 2005). The main feature of this method was the grafting of an immature scion on to a more mature stock before pollination.

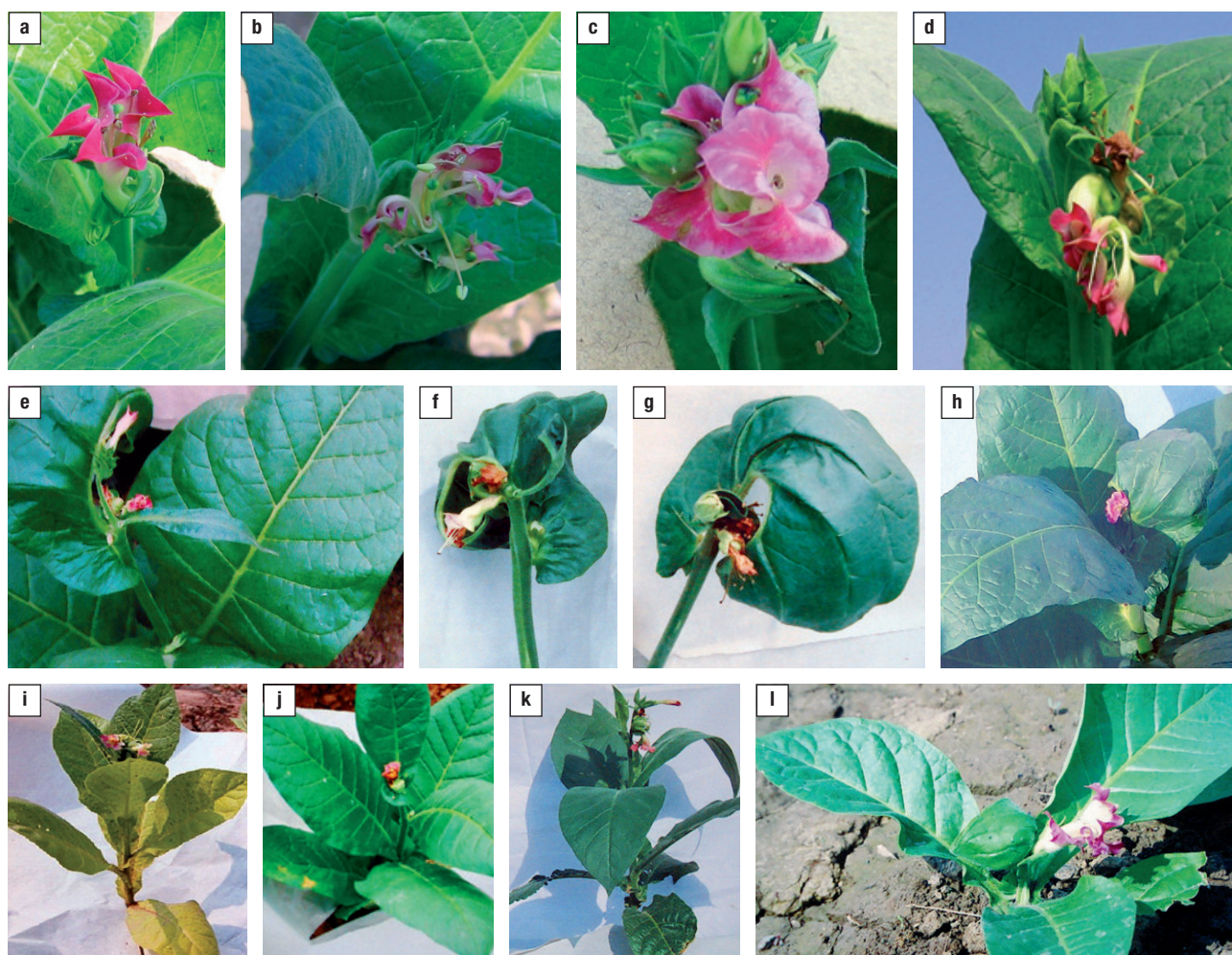


Figure 2. Representative variation of F₁ hybrids obtained from interfamilial hybridization between *N. tabacum* cv. 78-04 as pistillate parent and *Ocimum basilicum* L. as pollen parent. Some F₁ plants had only 1-4 flowers with altered morphology such as the short corolla and the exserted stigma (a, b, c, d) and part of them showed curling and twisting leaves with very dark green (e, f, g, h). Others had only 4-8 leaves and apparently dwarf, suggesting a shortened vegetative growth period and advanced flowering time (i, j, k, l).

Because the nutritional components offered by the rootstock were different from those existing in the scions, they might constitute a kind of stress to the scions (Zhang *et al.*, 2008). But between varieties and species, rootstocks might have different influence on scion' physiological metabolism or there might exist difference in affinity of every grafted combination. Despite the low seed set, a large number of unique interfamilial hybrids were still obtained using this method. Currently, the underlying molecular and biochemical mechanisms remain unknown, but this method can effectively introduce the genes of *Perilla frutescens* L. (Lamiaceae), *Mentha haplocalyx* Briq. (Lamiaceae), *Astragalus membranaceus* (Fisch.) Bge. (Leguminosae) and *Talinum paniculatum* (Jacq.)

Gaertn. (Portulacaceae) into *N. tabacum* (Wei *et al.*, 2010). *N. tabacum* is highly self-pollinating. Backcrossing is often used in cultivar development, and nuclear genes controlling disease resistance, morphological characteristics, or biochemical traits are frequently the focus of backcrossing (Lewis & Kernodle, 2009). Wei *et al.* (1998) study indicate that many germinating seeds were harvested from plants of both the BC₁ and open pollination of the F₁ hybrid.

Environmental factors can influence the crossability. A positive effect of high temperature in overcoming incompatibility and incongruity has been detected and applied in plant breeding by pollinating at high temperatures (Van Tuyl *et al.*, 1982; Okazaki & Murakami, 1992). But present study shows grafting followed by

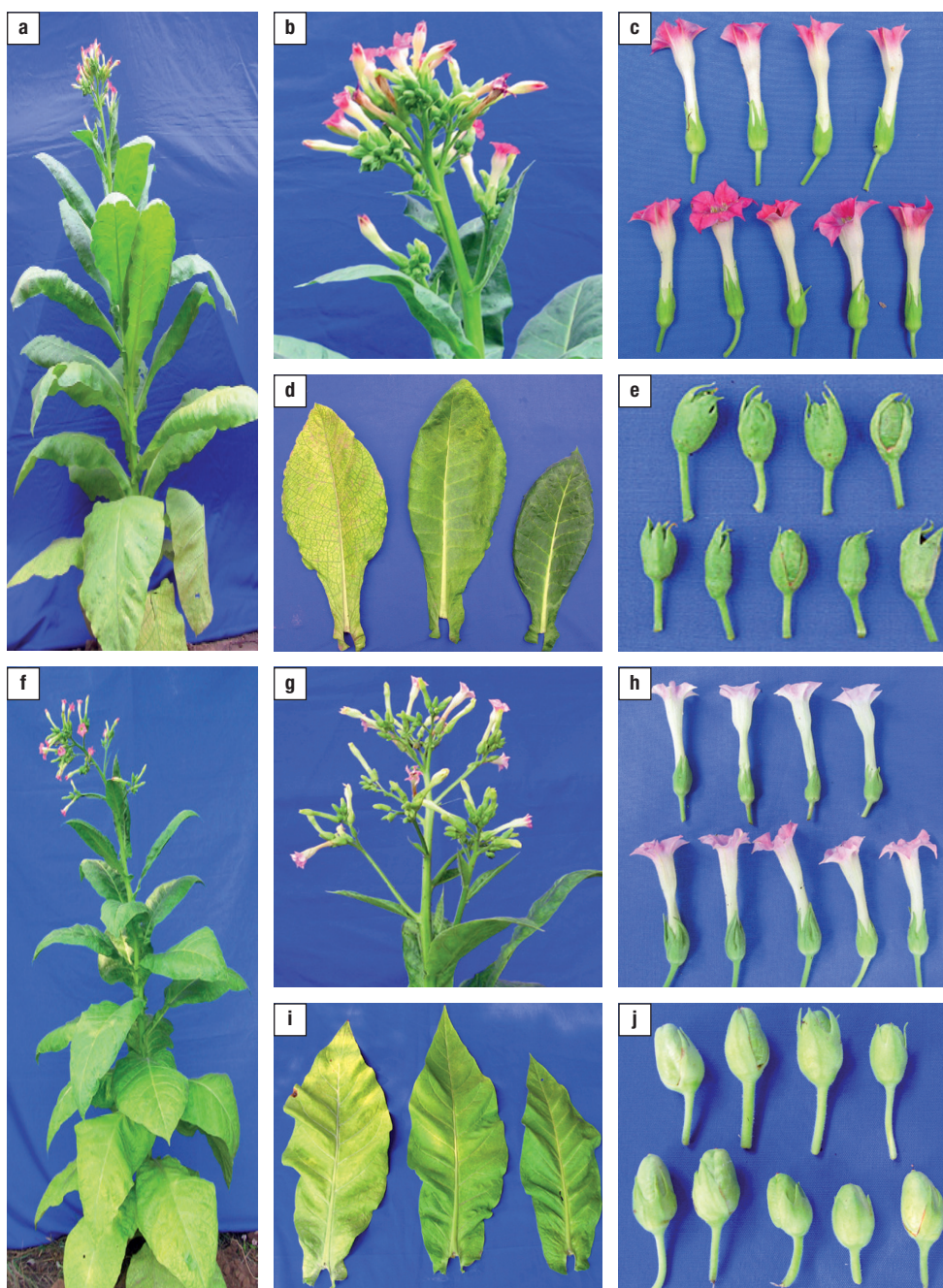


Figure 3. Phenotypic variation of several F_1 hybrids obtained from interfamilial hybridization between *N. tabacum* cv. 78-04 as pistillate parent and *O. basilicum* as pollen parent. Compared with 78-04, the plant type (f) and inflorescence (g) of several F_1 hybrids were similar to those of 78-04 (a, b), but the variations were distinct in flowers color (c, h), leaves shape and color (d, i) and capsules morphologies (e, j).

pollination at low temperature (22-25°C) and high relative humidity (70-80%) could facilitate gene exchange and recombination at the interfamilial level and efficiently overcome barriers of sexual incompatibility. In different combinations, the number of seeds per

capsule of the F_1 hybrids ranged from 0 to 426, which indicates that wide crosses also depend on the cross partners. Cytogenetic analyses have shown that chromosome number is heterogeneous in these hybrids (Chen, 2005; Li *et al.*, 2006).

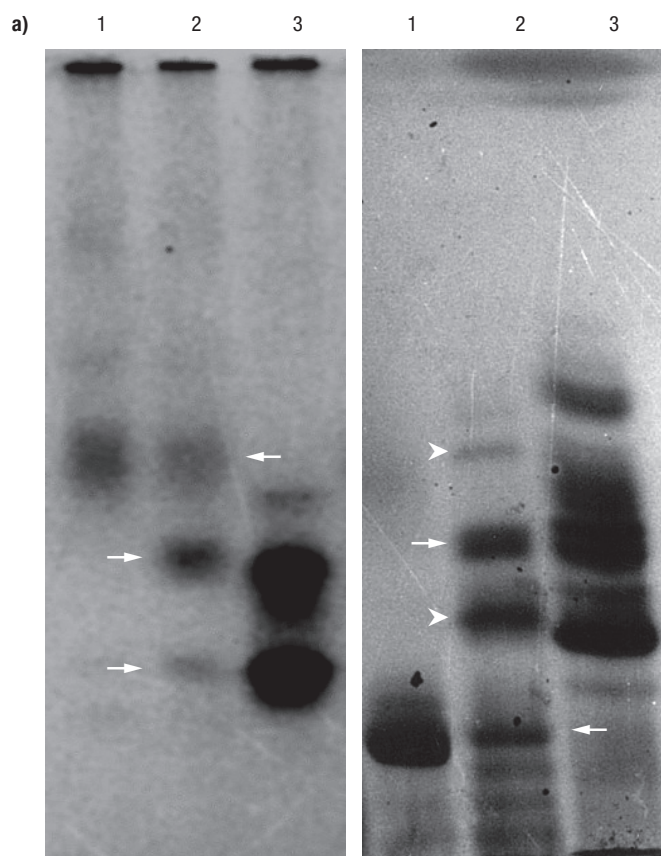


Figure 4. Representative isozyme profiles of F₁ hybrids and parents: (a) esterase isozyme zymogram, (b) peroxidase isozyme zymogram. Lanes: 1-78-04, 2-F₁ hybrid, 3-*O. basilicum*. Arrows indicate characteristic bands inherited from *O. basilicum* or 78-04, and arrowheads indicate new bands of the F₁ hybrid.

In recent years, genetic variability in *Nicotiana* increasingly gained attention because of investment in *Nicotiana* genomics research, interest in development of tobacco products with reduced harmful characteristics, and concentration on using *Nicotiana* species for plant-based production of commercially useful proteins (Lewis & Nicholson, 2007). Unlike most agronomic crops, tobacco is cultivated for its leaves rather than its reproductive parts. Some F₁ hybrid material displayed unfavorable agronomic traits in plant type, plant height, leaf size, leaf shape and number of leaves, etc. This might be a result of pleiotropic effects of the gene of interest *per se* or because of linkage drag effects caused by genes linked to the character of interest (Lewis & Rose, 2010). Research showed that a tremendous amount of phenotypic variability exists among strains of *N. tabacum*, but nucleotide variation, as revealed by RAPD is comparatively low. The major advantage of RAPD technology

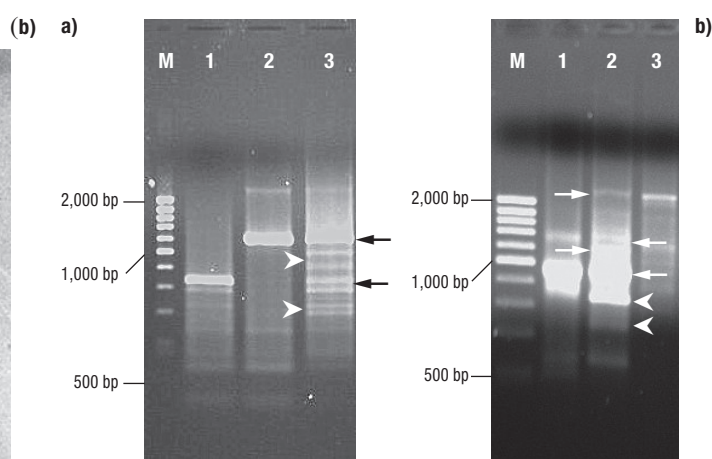


Figure 5. Representative RAPD profiles of F₁ hybrids and parents. RAPD profiles with (a) primer S28 (Lanes: M-100 bp DNA ladder, 1-78-04, 2-*O. basilicum*, 3-F₁ hybrid) and (b) with primer S265 (Lanes: M-100 bp DNA ladder, 1-*O. basilicum*, 2-F₁ hybrid, 3-78-04). Arrows indicate characteristic bands inherited from *O. basilicum* or 78-04, and arrowheads indicate new bands of the F₁ hybrid.

lies in exploration of large genomic portions without prior sequence information and requires small quantity of DNA. The bands which are specific for the pollen parent and occur in the hybrids are good markers to confirm the hybridity. Those non-parental bands may be generated from the differential gene expression in a different genetic background and may also be created by heteroduplex formation (Kiran *et al.*, 2009).

It is well-known that some plants, especially those belonging to the Lamiaceae family, possess a wide range of biological and pharmacological activities (Danesi *et al.*, 2008). *O. basilicum* has been cultivated from ancient times both as an ornamental plant and as a major essential oil crop. Although essential oils in different basil cultivars are variable, the prevalent chemical components are phenylpropanoids, such as estragole, eugenol, methyl-eugenol and methyl-cinnamate, and monoterpenes, such as linalool, geranial, neral and eucalyptol (De Masi *et al.*, 2006). Scientific studies have also shown that bioactive constituents in basil oil are antioxidant, anticancer, antimicrobial, antiviral, antifungal, repellent, insecticidal, or nematocidal (Tsai *et al.*, 2011). Many reports have been given about the agronomic performance of hybrids, but little or no information is available on chemical constituents of hybrids when compared with their parents. Our previous study indicated that the volatile compound of the hybrids, as assessed by gas chromatography-mass spectrometer (GC-MS), largely resembled that of the

parents (Wei *et al.*, 2008), which may be a favourable change in chemical quality. These components of *O. basilicum* are secondary metabolites produced under genetic regulation. This suggests that the biosynthesis pathway of essential oil of *O. basilicum* was transferred to the tobacco. Meanwhile, some new medicine and flavor components were found such as α -terpineol, famesene, and γ -gurjunene (Wei *et al.*, 2008).

In summary, our research indicates that pollination following grafting could efficiently overcome barriers of sexual incompatibility between *N. tabacum* and *O. basilicum* and introgress the valuable genes from *O. basilicum* into the gene pool of cultivated tobacco. The results will be of value in broadening the genetic basis of *N. tabacum* and creating new types of tobacco.

Acknowledgments

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