Flower Colour Inheritance in Nicotiana alata (Solanaceae) and its Use as a Genetic Marker for Gene Flow Studies

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***Abstract**: In Nicotiana alata, flower colour inheritance has followed Mendelian inheritance with dark colours being dominant over lighter colours. Reciprocal crosses concluded the absence of the cytoplasm involvement in the determination of flower colour. The backcross confirmed the dominant nature of red as the backcross between any F1 to the red parent produced only red flowers. On the other hand when light coloured morphs were the parents, the offspring segregated in a ratio of 1:1 confirming the monogenic recessive inheritance of light colours. The exception for the above patterns of inheritance was when the F1 of the lime green x pink was crossed with the pink pollen donor and produced red purple offspring. Flower colour in Nicotiana alata, could be used as an easily interpreted morphological marker in most morphs.*

Key words: Flower colour, inheritance, morphological marker, Nicotiana alata

INTRODUCTION

Gene flow and the subsequent hybridization and introgression of genes from cultivated and genetically modified (GM) crops into the gene pools of wild relatives has been documented in crops such as rice, rapeseed and maize. Transgene flow can occur via pollen-mediated gene flow or seed-mediated gene flow (Mallory-Smith & Zapiola 2008). There is a great concern about gene flow from GM crops to their close relatives because the transfer of transgenes could bring about unintended effects in natural ecosystems. For instance, Ellstrand, (2003) and Song et al. (2005) reported a case of rice whereby the gene flow from cultivated rice

contributed to the decrease in fertility of seed and pollen in wild rice (Oryza rufipogon) and probably led to the extinction of wild rice in Taiwan. Therefore, studies of gene flow are important in both conservation genetics (reviewed in Wan et al., 2004) and ecological risk assessment of GM crops (Ellstrand, 1999; Ellstrand, 2003; Snow et al., 2003).

Molecular markers, such as microsatellites and RFLPs, can be used for measuring genetic variation, gene flow and hybridization between populations and in determining paternity (Arias & Reiseberg, 1994; Jorgensen & Andersen, 1994; Morris et al., 1996; Paul et al., 1995). Molecular markers are widely used in genetic analysis because they are considered to be objective measures of variation as they concern the DNA molecule itself. They are not subject to environmental influences and tests can be carried out at any stage during plant development. However, they require complex equipment, expensive consumables and technical expertise (Khan & Spoor, 2001; Vicente & Fulton, 2003). Morphological markers can therefore be used as an alternative depending on the biological question under investigation. The advantage of morphological markers over molecular markers is that they do not require sophisticated equipment and provide a direct measurement of the phenotype (Vicente & Fulton, 2003). Morphological traits, such as flower, seed or seed-coat colours can be used as efficient genetic markers if they have simple inheritance (Cruzan, 1998). Flower colour has been used as a morphological marker for genetic studies in many species because of the ease of visual identification (Clarke et al., 2004; Kazan et al., 1993; Kumar et al., 2000).

Flower colour is either monogenically or polygenically inherited depending on the plant species. In several species such as Brasicca napus, B. rapa and Leucaena, flower colour is monogenically inherited (Chen & Heneen, 1990; Simioni et al., 1998; Rahman, 2001). In other species, however, flower colour appears to be determined by many genes, each with a small effect. For example, in Petunia over 30 genes are found to be involved in colour expression (Nieuwhof et al., 1990). Epistatic interactions between two or more genes, cytoplasmic effects and environmental factors may also play an important role (Pahlavani et al., 2004).

The inflorescence morphology of solanaceae is very complex (Lippman et al., 2008). For example, Kaczorowski et al., (2005) reported Nicotiana section Alatae to exhibit a great diversity among species in floral morphology which includes flower colour. Nicotiana alata (Link and Otto), a widely grown ornamental plant is found to occur in white, red,

pink, purple, green and yellow flower colour which can be used as a morphological marker. However, the genetics of flower colour inheritance in N. alata has not been extensively studied although in the related species N. langsdorffii and N. sanderae several genes are known to be involved (Smith, 1937). Our objectives were 1) to understand the mode of inheritance of flower colour in five different colour morphs (genotypes) of N. alata. 2) Identify flower colours that have monogenic inheritance which could be used as markers in studies of gene flow and hybridization in this species.

MATERIALS AND METHODS

We used five morphs (genotypes) of Nicotiana alata which differed in flower colour. The colours were white, lime green, pink, purple and red. Plants from true breeding lines of each morph were obtained from Åbergs trädgård, a nursery in southern Sweden. The plants were kept in the green house using ordinary potting soil and treated under ordinary agronomic conditions. The temperature in the greenhouse was kept constant at 23oC, with a light and a dark cycles of 12 hrs.

In the early summer of 2003 we performed controlled crosses between the colour morphs making a total of 20 cross combination including reciprocals (Table 1). For each cross combination we pollinated three flowers on each of the 20 recipient plants.

Flowers of the recipient parents were emasculated and bagged before anthesis, and after pollination flowers were again bagged to prevent contamination with pollen from undesired morphs. To determine the flower colour of the offspring, three capsules from each pollinated plant were taken and five seeds from each were grown in pots in the greenhouse. To avoid subjectivity, we scored flower colour using the Royal Horticultural Colour Chart (2001). To verify the genotype of the F1 and determine the number of genes that control flower colour from the segregation ratio, a backcross test was performed (Table 2). Backcrosses were made between F1s and both parents. Three crosses were made with each of the 20 F1 cross combinations, which gave us a total of 60 crosses. Twenty seeds from each capsule were sown in September 2004 for flower colour scoring.

RESULTS AND DISCUSSION

In this study we found that most flower colours in N. alata follow Mendelian inheritance and are controlled by a single locus with dark colours being dominant over lighter colours. Red flower colour was dominant over all colours, while white was recessive to all. Nineteen out of 20 monohybrid crosses produced either red or red purple flowers

(Table1). Differences in the intensities of flower colour between crosses of red x pink and lime green x pink and their reciprocals was observed. However, they were within the same colour category and were grouped together for statistical analysis. The crosses between white and lime green yielded yellow green flowers (Table 1).

Table1. Flower colour and number of F1 generation resulting from crosses among the five colour morphs

First crosses and reciprocals (♀ x ♂)	F1	Colour chart code	N
White x Red	Red purple	61B	15
Red x White	Red purple	61B	15
White x Purple	Red purple	67A	15
Purple x White	Red purple	67B	15
White x Pink	Red purple	73A	15
Pink x White	Red purple	73A	15
White x Lime green	Yellow green	145D	15
Lime green x White	Yellow green	145B	15
Red x Purple	Red	45C	15
Purple x Red	Red	45C	15
Red x Pink	Red	53C	15
Pink x Red	Red purple	N57A	15
Red x Lime green	Red	45B	15
Lime green x Red	Red	45B	15
Purple x Pink	Red purple	60B, 67A	8. 7
Pink x Purple	Red purple	60B, 67A	8. 7
Purple x Lime green	Red	53A	15
Lime green x Purple	Red	53A	11
Lime green x Pink	Red purple, Red	N57C, 53A	6, 6
Pink x Lime green	Red purple	61B, 60A	9, 6

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Since the reciprocal F1 crosses yielded the same results as the first crosses, it can be concluded that the cytoplasm is not involved in determination of flower colour in N. alata. The backcross confirmed the dominant nature of red as the backcross between any F1 to the red parent produced only red flowers. However, when the parents were recessive (light coloured) the offspring segregated in a ratio of 1:1 confirming the monogenic recessive inheritance of light colours in N. alata (Table 2). These results suggest that the F1s were heterozygous and that the allele's segregated during gamete formation as expected. Similar results have been reported of the dominant nature of violet flower colour over very light violet colour in Vigna sinensis (Hanchinal & Goud, 1978; Jindla & Singh, 1970). Also, results of a study by Agricultural Science Research Paper, (2012) to determine flavonoids show that Nicotiana alata don't contain the carotenoids,C3-OH hydrogen free of flavonols or chalcones, but they all contain aurones, and they have anthocyanins except the white flower. The purple has the highest content of anthocyanins, and it is the most important factor that controls the flower color. However, based on the general knowledge about the complexity of anthocyanin expression in plants (Forkmann, 1993) it cannot be excluded that additional genes are responsible for

differences in pink and red flower colours. The exception for the above patterns of inheritance in our study was when the F1 of the lime green x pink was crossed with the pink pollen donor and produced red purple offspring (Table 1 and 2). This ambiguous result could be due to the interaction between the pink and lime green colours as both were recessive when crossed with darker colours.

Table 2. Observed and expected ratios of flower colour segregation in backcross populations with chi – square values

Backcross F1 x Parents	Observed		Expected		Ratio	X2	P value
(White x Red) x Red	1W	58R	58R				
Red x White) x White	26W	33R	29.5W	29.5R	1:1	0.83	0.5
(White x Purple) x Purple	50P		50PP				
(Purple x White) x White	18W	21R	19.5W	19.5PP	1:1	0.23	0.9
(White x Pink) x Pink	1W	44R	45PK				
(Pink x white) x White	21W	29RP	25W	25PK	1:1	1.28	0.5
(White x Lime green) x Lime green	59LG		59LG				
(Lime green x White)x White	33W	24LG	28.5W	28.5LG	1:1	1.42	0.5
(Purple x Red) x Red	52R		52R				
(Red x Purple) x Purple	27R	33PP	30R	30PP	1:1	0.60	0.1
(Pink x Red) x Red	56R		56R				
(Red x pink) x Pink	25R	35PK	30R	30PK	1:1	1.60	0.1
(Lime green x Red) x Red	22R		22R				
(Red x lime green) x Lime green	32R	27LG	29.5R	29.5LG	1:1	0.42	0.5
(Purple x Pink) x Pink	53RP		53PP				
(Pink x Purple) x Purple	23PP	19RP	21PP	21PK	1:1	0.38	0.5
(Lime green x Purple) x Purple	60PP		60PP				
(Purple x Lime green) x Lime green	31RP	29LG	30PP	30LG	1:1	0.07	0.5
(Lime green x Pink) x Pink	45RP		22.5LG	22.5LG			
(Pink x Lime green) x Lime green	32R	28LG	30LG	30PK	1:1	0.26	0.5

(W= white, R= red, LG = lime green, PK = pink, PP = purple, RP = red purple)

According to our study, flower colour in Nicotiana alata, can be used as an easily interpreted morphological marker in the following cross combinations: red x white, red x lime green, lime green x white and pink x white. Some other crosses such as purple x pink and lime green x pink are not recommended because of the ambiguous results.

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References

Agriculture Science, (2012). The Research on the Major Genetic Traits in *Nicotiana Alata* Offsprings. http//www.agrpape.com.

Arias, D.M., and Reiseberg, L.H. (1994), Gene flow between cultivated and wild sunflowers. *Theoretical Applied Genetics*, 89: 655 - 660.

Chen, B.Y., and Heneen, W.K. (1990), Independent inheritance of flower colour and male-fertility restorer characters in *Brassica napus. Plant Breeding*, 104: 81 - 84.

Clarke, H.J., Khan, T.N., Siddique, K.H.M. (2004), Pollen selection for chilling tolerance at hybridisation leads to improved chickpea cultivars. *Euphytica*, 139, 65 - 74.

Cruzan, M.B. (1998), Genetic markers in plant evolutionary ecology. *Ecology*, 79: 400 - 412.

Ellstrand, N.C. (2003), Dangerous liaisons? When cultivated plants mate with their wild relatives. *Johns Hopkins University* Press, 244pp.

Ellstrand NC. 2003. Current knowledge of gene flow in plants: implications for transgene flow. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 358: 1163–1170.

Ellstrand, N.C., Prentice, H., and Hancock, J.F. (1999), Gene flow and introgression from domesticated plants into their wild relatives. *Annual Review of Ecology and Systematics*, 30: 539 - 563.

Forkmann, G. 1993: Genetics of flavonoids. pp 537–564 in: Harborne, J. (ed.) The Flavonoids: Advances in research since 1986. Chapman and Hall.ondon

Hanchinal, R.R., and Goud, J.V. (1978), Inheritance in *Vigna. Indian Journal of Genetics* 38, 339 - 342.

Jindla, L.N., and Singh, K.B. (1970), Inheritance of flower colour, leaf shape and pod length in cowpea (*Vigna sinensis* L.). *Indian Journal of Heredity*, 2: 45 - 49.

Jorgensen, R.B., and Andersen, B. (1994), Spontaneous hybridisation between oilseed rape (*Brassica napus*) and weedy B. *campestris* (Brassicaceae): A risk of growing genetically modified oil seed rape. *American Journal of Botany*, 81:1620 - 1626.

Kaczorowski, R.L., Gardener, M.C., Holtsford, T.P. (2005), Nectar traits in *Nicotiana* section *Alatae* (Solanaceae) in relation to floral traits, pollinators, and mating system. *American Journal of Botany*, 92 (8): 1270 - 1283.

Kazan, K.F Muehlbauer, J., Weeden, N.W., and Ladizinsky, G. (1993), Inheritance and linkage relationships of morphological and isozyme loci in chickpea. *Theoretical and Applied Genetics*, 86: 417-426.

Khan, S and Spoor, W. (2001), Use of molecular markers as a quality control in plant tissue culture. *Pakistan Journal of Biological Sciences,* 4: 479 - 482.

Kumar J.N. Vijayalakshmi, V.S., and Rao, T.N. (2000), Inheritance of flower colour in Chickpea. *The Journal of Heredity*, 91: 415 - 417.

Lippman Z. B., Cohen O., Alvarez J. P., Abu-Abied M., Pekker I., Paran I., Eshed Y., Zamir D.(2008). *The making of a compound inflorescence in tomato and related nightshades. PLoS Biol. 6, e288. (doi:10.1371/journal.pbio.0060288*.

Luby, L., and McNicol, R.J. (1995), Gene flow from cultivated to wild raspberries in Scotland: developing a basis for risk assessment for testing and deployment of transgenic cultivars. *Theoretical and Applied Genetic*, 90: 1133 - 1137.

Mallory-Smith C, Zapiola M (2008) Gene flow from glyphosate-resistant crops. Pest Manag Sci 64: 428–440.

Morris, W.F. Kareiva, P.M., and Raymer, P.L. (1996), Do barren zones and pollen traps reduce gene escape from transgenic crops? *Ecological Applications,* 4: 157 - 165.

Nieuwhof, M, Eijk, J.P., Garretsen, G., and Eikelboom, W.(1990), Inheritance of flower colour in relation to inheritance of flower pigments in tulip (Tulipa L.). *Journal of Genetics and Breeding*, 44: 277-280.

Pahlavani, M.H., Mirlohi, A.F., and Saeidi, G. (2004), Inheritance of flower colour and spininess in safflower (*Carthamus tinctorius L.). Journal of Heredity,* 95: 265 - 267.

Paul, E.M., Capiau, K., Jacobs, M., and Dunwell, J.M. (1995), A study of gene dispersal via pollen in Nicotiana tabacum using introduced genetic markers. *Journal of Applied Ecology* , 32: 875 - 882.

Rahman, M.H. (2001), Inheritance of petal colour and its independent segregation from seed colour in *Brassica rapa. Plant Breeding*, 120: 197 - 200.

Rieseberg, L.H., Kim, M.J., and Seiler, G.J. (1999), Introgression between the cultivated sunflower and sympatric wild relative, *Helianthus petiolaris* (Asteraceae). *International Journal of Plant Science*, 160: 102 - 108.

Simioni, C., Schifino-Wittman, M.T., and Paim, N. R. (1998), A model for flower colour inheritance in Leucaena (Leguminosae). *Genetics and Molecular Biology*, 21: 365 -368.

Smith, H.H. (1937), Inheritance of corolla colour in the cross *Nicotiana langsdorffii by Nicotiana sanderae. Genetics*, 22: 347 - 361.

Snow, A.A Pilson, D., Rieseberg, L.H, Paulsen, M.J., Pleskac, N., Reagon, M. R., Wolf, D.E., and Selbo, S. M. (2003), A Bt transgene reduces herbivory and enhances fecundity in wild sunflower. *Ecological Applications*, 13: 279 - 286.

Song ZP, Li B, Chen JK, Lu BR. 2005. Genetic diversity and conservation of common wild rice (Oryza rufipogon) in China. Plant Species Biology 20:83–92.

The Royal Horticultural Society (2001). *R.H.S. colour chart*. The Royal Horticultural Society, London.

Vicente, M.C., and Fulton, T. (2003), Using molecular marker technology in studies on plant genetic diversity. IPGRI and Cornell University New York

Wan, Q.H Wu H., Fujihara, T., and Fang, S.G. (2004), Which genetic markers for which conservation genetic issues? *Electrophoresi,s,* 25: 2165 - 2176.