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LINKAGE OF MORPHOLOGICAL MARKERS IN *BRASSICA*

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Abstract

Morphological markers play a pivotal role in selection of desirable traits in all plant breeding programs. The genetic linkage maps provide the basic information about the nature and place of genes on genetic maps. Two plant introduction (PI) germplasm of *Brassica napus* i.e. PI409024 and PI 535850 were interspecifically hybridized to 366 and 1203 lines of *B. campestris*. The hybrids i.e. 409024 x 1203 and crusher x 1203 were grown to produce F_1 generation. The F_1 populations were evaluated for genetic nature of four morphological qualitative traits like plant color, flower color, leaf shape and leaf pubescence at the KPK Agricultural University, Peshawar. In F_1 generation dark green plant color (C), dark yellow flower color (Y), entire leaf shape (E1) and non-hairiness of leaf (h) were expressed as dominant traits. In F_2 generation the hybrids segregated and were classified into their respective phenotypic classes. Linkages were detected between Y and H, and E1 and H, pairs of loci. The recombination frequency between Y and H loci was 17.7±10.3 cM, and between E1 and H was 32.3±9.9 cM.

Introduction

Polymorphic monogenic traits were some of the earliest genetic markers employed in scientific investigations and may still be optimal for genetic, breeding and plant germplasm management. Although morphological markers are limited in nature but their assays neither require sophisticated equipments nor complicated procedures (Singh and Singh, 1992). Monogenic morphological markers are generally simple, rapid and inexpensive to score (Ghafoor, 1999). Until recently scientific plant classification was based exclusively on morphological traits (Stuessy, 1999). Some of which may serve as genetic markers suitable for plant germplasm management (Gottlieb, 1984; Hilu, 1984). The amount of information provided by markers based approach depends on the type and number of markers and their linkage relationship (Singh & Singh, 1992).

Breeding of conventional oilseeds is a way to produce superior cultivars. One of the main objectives of any breeding program is to produce and release high yielding and improved quality lines for the farming community. Worldwide, oilseed Brassica ranks third after soybean and cotton on the basis of production and harvested area (FAO, 2007). It is one of the main sources of vegetable oil of the world. *Brassica* oilseed crops are grown annually on about 23 million hectares that provide over 36 million tones of the world's oilseed production (FAO, 2007). In Pakistan *Brassica* is the second most important source of vegetable oil after cotton seed. The objectives of the present study were to investigate the genetic basis of qualitative characters (plant color, flower color, leaf shape, and pubescence) and their validity in determining linkage for utilization in plant breeding program.

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Materials and Methods

Brassica napus lines viz., Crusher, Norseman, 409024, Salam, 535850, 158926, WW-15171, 535851 and 8966-1 of and *B. campestris* lines 366, 1203, 2065 and indigenous collection (from Rustam-Mardan and Cht-II of Khyber Pakhtunkhwa (KP) province Pakistan) were characterized for plant color "C", flower color "Y", leaf shape "E1" and leaf pubescence "H" (Table 1) at the KP Agricultural University Peshawar during 2006-07. Clean seeds these lines were provided by the Project "Development of Desi Sarsoon through conventional and modern techniques". Three *Brassica napus* lines i.e. 535850, 409024 and one public cultivar Crusher along with two parents of *B. campestris* i.e., 366 and 1203 were selected for crossing (Table 1) to get F_1 population. F_1 populations of the interspecific crosses were scored for morphological markers i.e. C, Y, E1, and H through visual observations. The F_2 material was shifted to Miadam-District Swat, Pakistan. Elevation of Miandam is about 850–1,170 m and is suitable for off-season cultivations. The experimental material was grown under natural field conditions i.e. neither fertilizers nor pesticides were applied in order to measure the full potential of the populations. At maturity, data was recorded for morphological markers in F_2 population on every individual plant. The letters used for different genes in this manuscript are the same as used in "Cruceferae: Compendium of Traits Genetics (Ginette Seguin-Swartz *et al*., 1987)". The data thus obtained were statistically analyzed using Linkage-1 software (Suiter *et al*., 1983).

Results

Interspecific crosses in *Brassica* are not very common because of the variation in chromosome number. The genotypes used in the present study were of diverse nature. In some cases two genes may be involved in controlling one trait. For confirmation of inheritance, all parents having contrasting traits were compared with F_1 and F_2 populations.

Plant color: *Brassica campestris* lines i.e., Rustam-Mardan, 366, 1203, 2065 and Cht-II have dark green plant color "C" whereas *B. napus* lines Crusher, Norseman, 535851, 409024, Salam, 535850, 158926 and 8966-1 have light green plant color "c" (Table 1). Plant color of F_1 crosses i.e., Crusher x 1203, and 366 x 535850 were C, while of cross 409024 x 1203 was light green or c (Table 2).

F_2 plants of crosses Crusher x 1203, and 366 x 535850 segregated into C and c phenotypes, the segregation ratio (9:3:3:1) was not in agreement with a dominant gene action. On the other hand, F_2 plants of the cross 409024 x 1203 was classified into c and C phenotypes. This variation is due to the diverse nature of parents and the said trait may be controlled by two epestatic genes. The F_2 population deviated 9:3:3:1 ratio that did not fit for goodness by χ^2 method.

Flower color: Among *B. napus* lines Crusher, Norseman, 535851, 409024 and Salam produced light yellow flowers "y" while line 535850, 158926 and 8966-1 produced dark yellow flowers "Y". All *B. campestris* lines also have dark yellow flower color "Y" (Table 1). Flower color of all the F_1 plants (Crusher x 1203, 409024 x 1203 and 366 x 535850) was also Y (dark yellow) which showed the presence of dominance genes, whereas y was recessive in *Brassica* (Table 2). All F_2 population of interspecific hybrids of *Brassica* deviated segregation in a 9:3:3:1 ratio for all the crosses which did not fit for goodness by χ^2 method.

Table 1. List of morphological markers in *Brassica*.

Species	Genotype	Plant color	Flower color	Leaf shape	Pubescence
Brassica napus	WW-15171	c	y	E1	H
	Crusher	c	y	E1	H
	Norseman	c	y	E1	H
	535851	c	y	E1	H
	409024	c	y	E1	H
	Salam	c	y	E1	H
	535850	c	Y	E1	H
	158926	c	Y	E1	H
	8966-1	c	Y	E1	H
B. campestris	Rustum Mardan	C	Y	e1	h
	366	C	Y	e1	h
	1203	C	Y	e1	h
	2065	C	Y	e1	h
	Cht-II	C	Y	e1	h

c: Light green, C: Dark green, y: Light yellow, Y: Dark yellow, E1: Lobed leaf shape, e1: Lyarate leaf shape, H: Glabrous, h: pubescent

Table 2. Summary of markers in inter- and intra-specific populations of *Brassica* of F_1 generation.

Crosses	Plant color	Flower color	Leaf shape	Pubescence
Crusher x 1203	C	Y	E1	H
409024 x 1203	C	Y	E1	H
366 x 535850	C	Y	E1	H

Leaf shape: Lobed and un-lobed leaf shape data was analyzed for inheritance in *Brassica*. All parents of *B. napus* were lobed "E1" whereas of *B. campestris* were unlobed "e1" (Table 1). The F_1 genotypes of crosses Crusher x 1203, 409024 x 1203 produced lobed leaves showing the presence of E1 genes (Table 2). The F_2 plants of both crosses segregated in to E1 and e1. These results show that the lobed trait is controlled by a dominant gene (E1). The F_2 population deviated segregation in a ratio of 9:3:3:1 for all the crosses which did not fit for goodness by χ^2 method.

Pubescence: All *B. campestris* lines had pubescence "H" whereas *B. napus* lines were glabrous "h" (Table 1). The F_1 crosses i.e., Crusher x 1203, 409024 x 1203 and 366 x 535850 were all h "non-pubescent" (Table 2). In F_2 generation all the crosses segregated for H and h. The presence of pubescence in crosses 2065 x 535851 and 1203 x 158926 (data not shown) was controlled by a dominant gene whereas the absence of pubescence in other crosses was recessive. This trait may be controlled by two epistatic genes. The F_2 segregation for these crosses did not show ratio that did not fit for goodness by χ^2 method.

Linkage analysis: Two or more genes said to be linked if they are located on the same chromosome. Different chromosomes also segregate independently during meiosis. Therefore, for two genes located at different chromosomes, it may be assumed that their alleles also segregate independently. The chance that an allele at one locus co-inherits with an allele at other locus of the same parental origin is 50% and such genes are called unlinked.

Table 3. Joint segregation for morphological markers of *Brassica* F_2 population for traits flower color, pubescence and leaf shape.

Crosses	Gene	A- B-	A- bb	aa B-	aa bb	Sum	χ^2	P	R ± SE
366 x 535850	Y: H	75	2	8	2	87	6.111	0.013	0.177 ± 0.103
Crusher x 1203	E1: H	62	14	2	2	80	2.368	0.124	0.323 ± 0.099

χ^2 : Chi-square, P: 0.05, R: Recombination value, SE: Standard error

Plant color was not linked to other traits. Linkage was observed between Y vs. H and E1 in 366 x 535850 cross. Recombination values between Y and H and Y and E1 were 17.7±10.3cM. In cross Crusher x 1203, E1 was linked with H and the recombination values between E1 and H were 32.3±09.9 cM (Table 3).

Discussion

All the qualitative characters revealed segregation independently and were fit to 3:1 Mendalian ratio for monogenic traits. Monogenic markers are useful in estimating the rate of out crossing in predominantly self pollinating crops. They also help in identification of F_1 hybrids in the breeding programs. Heterozygous are not possible to detect in case of complete dominance gene factors for morphological markers, therefore, the segregating ratios fit well in 3:1 chi-square ratio (Ghafoor *et al*., 2003). Dark green plant color was dominant over light green plant color in crosses Crusher x 1203, 366 x 535850 whereas light green plant color was dominant over dark green plant color in cross 409024 x 1203. These result can get great support from the finding of Sampson (1966), who studied that the gene *pg*-1 for pale green foliage (pale true leaves and dwarfness) in broccoli [var. italica] was completely recessive and linked to gene *cr* (cream petal) with 11.0 ± 0.8% recombination. The gene *pg*-2 for pale green foliage (yellowish cotyledons, true leaves paler than in *pg*-1 plants, reduced vigor and delayed flowering) was completely recessive. Dark green plant color was dominant in most of the crosses. These results were found similar to those of Kianian & Quiros (1992a), who reported segregation ratios in F_2 populations (46:14 and 41:19 dark green to light green plants) derived from a cross between commercial Chinese kale [var. alboglabra] accessions with dark green foliage (B479) and light green foliage (B478) fitted to a dominant monogenic model.

Dark yellow flower color was observed to be dominant over light or cream yellow flower color. Getinet *et al*., (1993) got the same results in reciprocal crosses between line PGRC/Ethiopia Crusher 24 (cream petals) and true-breeding yellow-petal led line Awassa selection 67 to produce F_1, F_2, and BC_1 generations. All F_1 reciprocal hybrids (151 hybrids) and backcrosses to the yellow-petalled parent (1,195 plants) had yellow petals, indicating dominance of yellow over cream. Backcrosses to the cream-petalled parent segregated in a 1:1 ratio (418 yellow: 398 cream). The F_2 generation segregated in a 3:1 ratio (349 yellow: 135 cream), indicating monogenic control, with the yellow petal allele dominant over the recessive cream petal allele. Lobed leaf shape is observed in the hybrids Crusher x 1203, 409024 x 1203, 366 x 535850 which indicated the dominance of the lobed leaf shape and the lyrate leaf shape was found to be recessive. Our results are supported by the studies of Klein Geltink (1983) as they studied crosses between turnip rape [subsp. oleifera] cv. Goldwalze (entire leaves) x cvs. Krasnaja Samarkandskaja and Tsutsui (cut leaves), and in crosses between cv. Teutonengold x KL 1 (entire leaves) and cv. Krasnaja Samarkandskaja (cut leaf), the F_1 plants had entire leaves. F_2 progenies segregated in a 3:1 ratio of plants with entire leaves to plants with cut leaves, indicating a

single dominant gene. In the cross KL 1 x cv. Krasnaja Samarkandskaja, a digenic inheritance was obtained for the F_2 population, i.e., 15:1 plants with entire leaves to plants with cut leaves. The genotypes for these cultivars were postulated as being E1E1e2 e2 or e1e1E2 E2.

In some of our crosses, pubescence or hairiness trait was dominant over non-hairiness or glabrousness. Mohammad & Sikka (1937) found that pubescence was dominant over glabrousness with a 3:1 ratio, indicating single gene control. Thompson (1956) reported that hairiness of the first leaf, was found to be dominant in marrow stem kale (var. acephala). The crosses Crusher x 1203, 409024 x 1203, 366 x 535850 were non-pubescent or glabrous. Beck *et al*., (1975) studied the inheritance of glabrous vs. pubescent leaf type in *Crambe hispanica* USDA Plant Introduction (279346). Two glabrous genotypes were found in this normally pubescent accession. One genotype segregated with respect to leaf type and a second one produced all glabrous leaves. Six individuals from the former plant **w**ere self-pollinated. Selected plants were paired randomly with other *Crambe hispanica* types and crossed reciprocally. Combined segregation ratios in 44 F_2 families involving 4,004 seedlings (3,037 glabrous: 967 pubescent) indicated that the glabrous trait was controlled by a single gene "P". Plants with glabrous leaves were postulated to have the dominant allele "P" and plants with pubescent leaves, the recessive allele "p". Results were verified in 155 F_3 generation populations (derived from selling of randomly selected glabrous F_2 plants) which fitted to the expected 1:2 ratio (54:101); all 44 pubescent F_2's produced pubescence in F_3.

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