

HERITABILITY STUDIES FOR SEED QUALITY TRAITS IN INTROGRESSED SEGREGATING POPULATIONS OF *BRASSICA*

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Abstract

Estimation of genetic parameters in the context of trait characterization is an essential component of future targeted crop improvement programs. Collection of knowledge about genetic behavior such as genetic variability and heritability etc., of the germplasm is the basic step for initiation of any breeding program. Genetic variability and Broad sense heritability for various seed quality traits in 10 brassica genotypes and their 12 F₂ progenies comprising of introgressed hybrids were studied. The genotypes had highly significant variation for oil content, protein, glucosinolates contents, oleic, linolenic and erucic acid contents. Glucosinolates content and erucic acid showed high heritability in all F₂ populations, while rest of the traits showed variable trends. The cross combination 547 x 118 (*B. napus* x *B. campestris*) proved to be a good interspecific hybrid that had high proportion of introgression and has high heritability for beneficial traits. The individual plants having combination of desirable traits were also identified from the F₂ populations.

Introduction

Modern crop breeding has resulted in the reduction of genetic variability in most of the crop species (Tanksley & Nelson 1996). Inter-specific hybridization has been widely used to increase genetic diversity and to introgress desirable traits from one gene pool to another genetic background, but often, inter-specific crosses are unsuccessful. Certain factors such as low rates of recombination and limited chromosome homology can make introgression of the target trait a difficult task to achieve (Brown *et al.*, 2003; Desloire *et al.*, 2003). Extensive genetic mapping in the genus Brassica has led to the availability of a number of tools to detect the transfer of traits between different species. The morphology of leaves, flowers, and pods has been successfully used to distinguish the species of the Brassicaceae (Nawaz *et al.*, 2013; Gomez-Campo, 1980), but these morphological traits may sometimes be vague for determining true hybrids, because these may often have more similarity to one of the parents than to the expected intermediate phenotype. Primary goal of plant breeder is to achieve improved yields, nutritional qualities, and other traits of commercial value (Ali *et al.*, 2013; Moose & Mumm, 2008). The plant breeder usually keeps such ideal plants in mind that combines a majority of desirable characteristics. These may be general and/or specific traits that could contribute to the improved adaptation of plants to the environment and could enhance yield with better quality seed. In order to achieve the objectives and combine desirable genes found in two or more different varieties, planned hybridization between carefully selected parents is practiced within or among species. For this purpose knowledge of genetic diversity is indispensable in the development of commercial hybrids (Ahmad *et al.*, 2013). Introgressive hybridization or introgression involves gene flow from one species to another by repeated backcrossing of an interspecific hybrid with one of its parent species. It is a source of speciation in crop species. In case of land races, where open pollination is a case; such plants

may be observed that have phenotypic characters of both parents. Partial hybrids with female-parent-type phenotypes and chromosome numbers but altered genomic compositions have been reported in wide crosses of several plants (Tu *et al.*, 2009). High oil yield and low levels of undesirable compounds is an ultimate goal of oilseed breeder. Introgressive hybridization may lead to the production of plants with high oil yield, which may be used for the production of elite varieties, and ultimately the gap between the production and import of oil may be lessened. Keeping all of the perspectives in mind, the present study was conducted to investigate the heritability of seed quality traits in the F₂ populations comprising of plants that had introgressed morphological traits of distant parental species altogether.

Materials and Methods

The present study was conducted in the experimental fields of the University of Agriculture, Peshawar. For this study, eight *B. napus* accessions viz. 502, 510, 514, 525, 531, 532, 533, 547 locally collected from different areas of Khyber Pakhtunkhwa and Punjab provinces were crossed with two male parental lines i.e. 109 and 118 representing *B. juncea* and *B. campestris*, respectively in line x tester fashion (Table 1). Hence 16 crosses were developed. Out of these 16 F₁ crosses, 12 hybrids showed introgression (some plants had morphological characteristics of both of their respective parental species). The qualitative traits like leaf base, pod shape and angle, flower shape and color were used to identify the plants having combined traits of both parental lines defining distinct species. Single introgressed F₁ plant was selected from each cross and was selfed to produce F₂ populations. Seed of the parental accessions and F₂ progenies were space planted in the field in randomized complete block design with three replications. The F₂ seeds were space planted in lines maintaining plant to plant distance of 10 and row spacing of 60 cm. All cultural practices were provided and introgressed hybrids in the segregating populations

of 12 crosses were identified. Morphological data were recorded on 10 plants from each parental line and 40 introgressed hybrids from each F₂ population. At maturity, seeds were analyzed for seed quality traits viz. oil content, protein content, glucosinolates contents, oleic acid, linolenic acid and erucic acid.

Data for all these attributes were subjected to analysis of variance following Steel & Torrie (1980) in order to evaluate the significant differences in seed quality traits of the studied genotypes. Heritability in broad sense was calculated according to the formula suggested by Mahmud & Kramer (1951) and heritability values were categorized according to Robinson *et al.*, 1949).

Table 1. Species and collection site of *Brassica* accessions used in this study.

S #	Accession code	Species	Collection site
1.	502	<i>B. napus</i>	Rawalakot
2.	510	<i>B. napus</i>	Okara
3.	514	<i>B. napus</i>	Rajanpur
4.	525	<i>B. napus</i>	Khairabad
5.	531	<i>B. napus</i>	Diamer
6.	532	<i>B. napus</i>	Rawalpindi
7.	533	<i>B. napus</i>	Swabi
8.	547	<i>B. napus</i>	West haripur
9.	109 (UCD-40)	<i>B. juncea</i>	USA
10.	118 (RL-16)	<i>B. campestris</i>	Islamabad

Results

Mean squares revealed highly significant differences among F₂ progenies and their parents for all studied seed quality (Table 2).

The estimates of mean, variance, and heritability for seed quality traits in F₂ population and their respective parents are presented in Tables 3 and 4. It is evident from these Tables that the cross combination 547 x 118 (*B. napus* x *B. Campestris*) had the maximum (44.3%) OC, while it was the minimum (36.1%) in the cross 502 x 109 (*B. napus* x *B. juncea*). (Table 3). Average oil content (OC) % in parental line ranged from 42.7% (502) to 49.4% (525). The highest heritability was observed in the crosses 533 x 118 and 502 x 109 (0.97). The F₂ means for protein content showed deviation from

their respective parents but remained mostly within the parental range, while heritability ranged from medium to high in the F₂ populations (Table 3). The striking feature of introgressed hybrids in all of the 12 populations were low levels of glucosinolates in F₂ populations within them as compared to their parents. Hence introgressed hybrids behaved far better than normal F₂ hybrids for this trait. Glucosinolate content (GS) ranged 69.3-134.5 μ Mg⁻¹ in parental lines, while in F₂ populations varied from 53.8 to 95.5 μ Mg⁻¹. Variance in F₂ populations was also high which led to high heritability values in all of the F₂ populations (Table 3). Oleic acid (OA) is a monounsaturated fatty acid found in excessive amount (50 to 70 %) in canola oil. It is nutritionally beneficial for human health. In the present study, small differences were found in the means for OA in both sets of populations. High variability was observed for oleic acid in all F₂ populations. The highest level (55.3%) of oleic acid (OA) was observed in *B. napus* parental line (547) while the *B. juncea* parental line (109) had the lowest OA contents (29.9%). The cross 547 x 118 had the highest percentage of OA same as that of its female parent (55.3%) while the lowest value (48.1%) was exhibited by two cross combinations 532 x 118 (*B. napus* x *B. campestris*) and 525 x 118 (*B. napus* x *B. campestris*). Linolenic acid is (LA) a *w*-3 polyunsaturated fatty acid which may have some nutritional benefits, but due to the occurrence of three double bonds, it gets oxidized quickly, thereby decreasing the shelf life of oil. 3-5% level of this fatty acid is desirable (Mead, 1977). Mean values of F₂ populations for linolenic acid fell within the range of the parental genotypes. In parental lines, a range of 9.3% (547) to 13% (109) was recorded. The cross combination 547 x 109 had the desirable levels (5.1%) of LA, whereas the level of quantity was maximum (9.3%) in the cross 547 x 118 (Table 4). The heritability values for LA showed variable trends in all F₂ populations. Erucic acid has many applications in petroleum industry. This long-chain fatty acid is not desirable in oil for human consumption (Ofori *et al.*, 2008). In the current study, parents as well as F₂ populations showed high levels of EA (Table 4). High variance in F₂ populations led to high heritability estimates for EA in all 12 F₂ populations (Table 4).

Table 2. Mean squares due to different sources of variation for oil content, protein content, glucosinolates content, oleic acid, linolenic acid, erucic acid for parents and crosses.

Source of variation	df	Oil content	Protein content	Glucosinolates	Oleic acid	Linolenic acid	Erucic acid
Reps	2	1.66	3.84	132.39	0.038	1.20	20.93
Genotypes	21	30.52**	63.71**	833.82**	95.99**	16.24**	252.91**
Error	42	2.95	1.66	67.02	2.55	0.79	12.11
C.V (%)	--	3.95	4.95	10.10	3.29	11.28	7.79

** = Significant at 1% level of probability

C.V. = Coefficient of variation

Table 3. Mean, variance and heritability estimates for some seed quality traits of parents and crosses.

Genotype/cross	Oil content			Protein content			glucosinolates		
	Mean	δ^2	h^2	Mean	δ^2	h^2	Mean	δ^2	h^2
533	47.4	0.57	--	22.4	0.19	--	73.5	0.39	--
532	47.3	0.21	--	23.9	3.04	--	72.9	1.63	--
118	46	0.39	--	25.3	0.49	--	102.5	4.57	--
514	45.1	6.07	--	45.8	2.77	--	69.3	5.08	--
547	44.3	0.07	--	26.7	0.57	--	79.5	1.47	--
502	42.7	1.39	--	27.2	0.79	--	71	2.01	--
510	43.8	2.47	--	25.6	1.29	--	91.8	0.76	--
525	49.4	0.8	--	23.4	0.6	--	85.9	4.5	--
531	48.1	0.37	--	24.9	2.73	--	87.4	0.49	--
109	43.9	3.0	--	26.2	0.2	--	134.5	6.3	--
533 x109	42.1	10.8	0.88	25.1	3.8	0.94	83.3	216.8	0.99
533 x 118	36.2	19.9	0.97	27.4	20.9	0.98	56.1	715.1	0.99
532 x 109	43.1	5.7	0.86	24.9	2.8	0.71	72.8	75.4	0.95
532 x 118	43.	8.0	0.96	24.2	3.0	0.59	70.5	501.6	0.99
514 x 109	43.1	8.0	0.46	25.4	7.1	0.89	78.2	155.9	0.96
514 x 118	42.4	2.2	0.28	25.4	2.8	0.58	84.1	343.0	0.98
547 x 109	43.1	10.0	0.95	21.7	2.6	0.86	53.8	302.3	0.98
547 x 118	44.3	15.0	0.98	26.7	4.0	0.86	79.5	506.6	0.99
502 x 109	36.1	80.9	0.97	24.8	12.8	0.96	63.7	64.4	0.94
510 x 109	43	13.6	0.79	25.5	2.3	0.77	82.3	124.9	0.98
525 x 118	41.4	4.9	0.88	26.3	1.9	0.70	95.5	66.0	0.93
531 x 118	42.9	17.48	0.97	25.5	53.67	0.97	79.6	24.24	0.94
LSD (0.05)	2.83	--	--	2.12	--	--	13.49	--	--

 δ^2 =Variance, h^2 =heritability (BS)**Table 4. Mean variance and heritability estimates for some seed quality traits of parents and crosses.**

Genotype/cross	Oleic acid			Linolenic acid			Erucic acid		
	Mean	δ^2	h^2	Mean	δ^2	h^2	Mean	δ^2	h^2
533	51.5	0.27	---	9.4	0.49	--	49.4	1.33	--
532	51.6	1.17	---	9.8	0.71	--	49	0.37	--
118	39.3	0.52	---	10.7	1.63	--	56.9	0.48	--
514	44.6	4.89	---	9.6	1.39	--	59.5	1.33	--
547	55.3	0.21	---	9.3	0.67	--	44.4	0.43	--
502	52.6	0.67	---	10	1.81	--	48.3	0.13	--
510	51.7	4.71	---	9.8	1.33	--	51.2	0.25	--
525	43.2	0.2	---	9.7	1.1	--	60.4	0.8	--
531	50.1	0.19	---	10.4	0.67	--	50	1.83	--
109	29.9	3.0	---	13	0.2	--	60.4	0.8	--
533 x109	50.4	18.3	00.95	6.1	2.9	0.89	37.8	22.0	0.95
533 x 118	49.7	2.3	00.84	5.9	1.8	0.49	28.2	116.9	0.99
532 x 109	51.8	21.7	00.91	7.4	0.67	0.45	35.3	66.1	0.99
532 x 118	48.1	7.6	00.89	5.7	4.3	0.61	36.5	103.6	0.98
514 x 109	52.4	10.2	00.62	5.7	1.7	0.69	37.9	20.1	0.94
514 x 118	53.4	9.0	00.82	5.9	1.2	0.21	35.9	26.6	0.97
547 x 109	51.9	15.5	00.94	5.1	0.6	0.43	35.1	48.7	0.98
547 x 118	55.3	53.5	00.99	9.3	0.8	0.39	44.4	63.3	0.95
502 x 109	49.5	8.1	00.82	6.3	0.7	0.12	42.9	5.2	0.93
510 x 109	48.7	5.8	00.34	6.9	0.7	0.31	45.8	16.4	0.97
525 x 118	48.1	4.0	00.92	7	0.4	0.49	44.2	6.9	0.91
531 x 118	43.3	11.03	0.97	4.5	6.3	0.83	33.7	33.02	0.97
LSD (0.05)	2.6	--	--	1.46	--	--	5.73	--	--

 δ^2 =Variance, h^2 =heritability (BS)

Discussion

Highly significant differences for all the traits suggested the presence of sufficient genetic variability in the introgressed hybrids. Hence it was proved that this selection strategy may prove to be a useful source for providing promising material in the future breeding program. The results of the present study are supported by the findings of Khan *et al.*, (2008) who reported highly significant results for glucosinolates (GS), oleic acid (OA), linolenic acid (LA) and Erucic acid (EA) in F_{3,4} *Brassica* populations. Similar results are reported for GS, OA and EC in rapeseed (Inayt *et al.*, 2009) and for GS among brassica lines (Bradshaw & Wilson 1998; and Krzymanski, 1999). Our results are in agreement with that of Chauhan & Tyagi (2002) who reported significant differences for EC in Indian mustard. The results revealed that F₂ introgressed progenies showed a considerable increase in variation from their respective parents for the studied traits and hence appeared to be a source for providing a unique set of *Brassica* germplasm that had a mixture of beneficial traits from both parental species. The findings of Ghosh and Gulati (2001), Khan *et al.*, (2006) and (Dar *et al.*, 2010) supported our results. They reported similar range of heritability for OC. Aytac & Kinaci (2009) reported moderate heritability for oil and protein content. Glucosinolate, sulphur containing compounds are undesirable for oilseed industry. Introgressive hybridization had been practiced in the past to improve meal quality of *Brassica* species. For this, genes controlling low glucosinolate (GS) were introgressed into *B. rapa* from *B. napus* during the late 1960's (Krzymanski 1970), which drastically increased the economic value of *Brassica* as oil crop. Khan *et al.*, (2008) observed high heritability for LA in F_{3,4} populations of *Brassica* species. Higher heritability for OA and LA found in some cross combination is supported by Schierholt & Becker (2011) while studying the inheritance of oleic acid in *B. napus*. They suggested that the higher heritability might be a cause of environmental influence that can be reduced through biparental or pedigree selection. Zhang and Zhou (2006) reported lower heritability for OA. Higher heritability values for OA and have also been reported by Schierholt & Becker (2011), who studied inheritance of OA in *B. napus*. They reported that monogenic inheritance is involved for high heritability of the trait.

It is commonly observed that the *Brassica* species cultivated in India and Pakistan have as much as 40-50% erucic acid and high glucosinolate (80-160µm g⁻¹) (Agnihotri & Kaushik, 1999). Chauhan & Tyagi (2002) also reported high heritability for EA.

Conclusions

Ultimate goal of oilseed breeder is to increase the oil quantity and quality of *Brassica* species. It is a common observation that parental species are high in oil content than that of successive generation on mean basis. But on

individual level, such plants may be present within preceding populations that have best performance than their parental species. In the present study, single plants having combination of desirable traits were identified, the incorporation of these plants in the future breeding program may help in the development of elite varieties.

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