

Heritability and genetic advance for effective selection in opium poppy (*Papaver somniferum*)

BRIJ K MISHRA¹, SUDHIR SHUKLA², ANU RASTOGI³ and ANKITA SHARMA⁴

National Botanical Research Institute, Lucknow, Uttar Pradesh 226 001

Received: 28 April 2008; Accepted: 28 March 2010

ABSTRACT

A study was conducted during 2003–08 to examine the genetic gain and heritability for 5 quantitative traits in different generations of opium poppy (*Papaver somniferum* L.). For the formulation of effective selection strategy in opium poppy (*P. somniferum* L.), cyclical process of testing selections, discarding inferior genotypes and retesting the superior selections, i.e. multistage testing is an essential step for its evaluation. The progenies of randomly selected individuals from 14 promising hybrids were evaluated over F₂ to F₆ generations for opium and seed yield and their contributing traits, viz capsules/plant, capsule size (cm²), seed yield/plant (g), opium yield/plant (mg) and morphine content. In general heritability and genetic gain declined from generation to generation. A cross between 'MOP 541' × 'BR 241' showed similar pattern for genetic gain in all the traits. The values of broad sense heritability decreased from F₂ to F₆ generation for most of the traits.

Key words: Genetic gain, Heritability, Multistage testing, *Papaver somniferum*

Multistage testing of strains (lines, hybrids, clones etc.) is an essential aspect in plant breeding programmes. Multistage testing, here refers to many generations of crop evaluation (the cyclical process of testing selection, discarding inferior genotypes and retesting the superior selections) until it reaches to stability with desirable characteristics and high level of productivity. Periodic evaluation of genetic improvement is essential for understanding yield-limiting factors, illustrating the importance of plant breeding to the public and identifying the traits that might require increased efforts by breeders (Evans 1993). Apart from this, genetic gain and heritability (in broad sense) at each generation must be examined so that selection may be intensified at a particular generation and the inefficient generation can be discarded (Ahmed *et al.* 2006). This programme is quite expensive from the economic point of view for the breeders and farmers. Very few researchers attempted a comprehensive examination of multistage system of test in Opium poppy (*Papaver somniferum* L.). Since Opium poppy is one of the most important medicinal plant due to its valuable alkaloids, especially for morphine in latex. Poppy seed is also in demand as it possesses high content of protein (24%) with large amount of edible oil (up to 50%) rich in linoleic acid (70%)

which lowers the cholesterol level in the blood of human system (Singh *et al.* 1995). For the last few decades, a number of high-yielding varieties have been developed (Shukla and Singh 2004). However, development of new high-yielding varieties is a continuous process of plant breeding. Hybridization is often used to generate sufficient level of genetic variability, other desirable characteristics and to obtain segregating population with high productivity (Ceolin *et al.* 2007). Generally, in the segregating population of opium poppy the probability of obtaining individuals with all the favourable alleles through subsequent generations is reduced. Opium yield is the most important trait in selective process and it can be enhanced through indirect selection of agronomic traits that are positively correlated with productivity (Cruz and Carneiro 2003). Prior to the release of a variety for commercial cultivation, it must be tested for several generations. The objective of the present study was to examine genetic gain and heritability for 5 quantitative traits in different generations of opium poppy and to determine ways to increase the overall gain, if possible.

MATERIALS AND METHODS

The experimental material consisted of 14 promising crosses which were evaluated from F₂ to F₆ generations in randomized block design with 3 replications each with 2 rows of 3 m length at the field of National Botanical Research Institute, Lucknow located at 26.5°N, 80.5°E and 120 m

^{1,4}Project Assistant II, ²Senior Scientist, ³Senior Research Fellow, Department of Genetics and Plant Breeding

above sea level. The plant-to-plant spacing was 10 cm and row-to-row was 30 cm. During the field experimentation 10–15 tonnes/ha farmyard manure and inorganic fertilizers @ 90 kg N + 60 kg P₂O₅ + 60 kg K₂O/ha was applied. Half of the nitrogen and full phosphorus and potassium fertilizers were used as basal dressing at the time of last ploughing. Remaining half of the nitrogenous fertilizer was applied as top-dressing in 2 splits after second and fourth irrigation. Ten irrigations were given during the crop season depending upon the soil moisture conditions. Two to three times prophylactic sprays of Dithane M-45 (0.2%) were given for protection of the crop due to disease (Yadav *et al.* 2006). The study is based on 14 F₂ crosses and their subsequent generation which were advanced following selfing of randomly selected plants. The crosses were selfed to produce F₃ and subsequent generations. In F₃ and subsequent generations each cross was grown in first week of November with 3 replications during 2004–05 to 2007–08. Ten plants/replication were selected individually in each cross and selfed. The selfed seeds of 10 plants were pooled to obtain the next generation. The data for capsules/plant, capsule size (cm²), seed yield/plant (g), opium yield/plant (mg) and

morphine percentage were recorded on 10 randomly selected plants of each replication in all the generation under study. Opium latex was collected from fully grown green capsules and subsequently seeds were harvested from the dried capsules. Morphine percentage in opium was determined following the method of Pride and Stern (1954). The mean values of data for all the 5 traits were used for the analysis. Genetic gain was calculated by using broad sense heritability as described by St. Martin and Futi (2000). Expected genetic gain over F₂ base population was obtained by the expression

$$GG = (X_s - X_o).H^2$$

$$GG\% = (GG/X_o).100$$

The broad sense heritability (H²) or the degree of genetic control was calculated for each trait using formula

$$H^2 = s^2_g / (s^2_g + s^2_e)$$

RESULTS AND DISCUSSION

The mean data of promising 14 crosses for different traits over F₂ to F₆ generations are presented in Table 1. The analysis of variance for each trait indicated that genotypic mean squares for all the traits were highly significant which suggested significant amount of phenotypic variability among

Table 2 ANOVA for 5 traits over 6 generations (F₂ to F₆) in opium poppy

Crosses/sources	Generations	Treatments (df 13)	Replication (df 2)	Error (df 26)	s ² _g	s ² _p	σ ² _e	Heritability
Capsule number /plant	F ₂	1.018**	0.006	0.012	0.335	0.348	0.012	0.963
	F ₃	0.702**	0.108	0.277	0.141	0.234	0.094	0.604
	F ₄	0.303**	0.015	0.148	0.051	0.200	0.148	0.258
	F ₅	0.731**	0.003	0.123	0.202	0.325	0.123	0.622
	F ₆	2.017**	0.113	0.499	0.506	1.005	0.499	0.503
	Capsule size(cm ²)	F ₂	2.163**	0.193	0.200	0.654	0.854	0.200
F ₃		5.662**	0.980	2.013	1.216	1.887	0.671	0.644
F ₄		5.925**	1.443	2.185	1.246	3.432	2.185	0.363
F ₅		9.674**	0.128	0.651	3.007	3.659	0.651	0.821
F ₆		11.202**	1.342	2.108	3.031	3.631	2.108	0.589
Seed yield/plant(g)		F ₂	4.014**	0.245	0.472	1.180	1.653	0.472
	F ₃	4.412**	0.133	1.116	1.098	1.470	0.372	0.747
	F ₄	4.974**	0.298	2.023	0.983	3.006	2.023	0.327
	F ₅	6.245**	2.263	0.713	1.843	2.557	0.714	0.720
	F ₆	7.194**	0.212	1.850	1.781	3.631	1.850	0.490
	Opium yield/plant (mg)	F ₂	4746.663**	68.839	68.78	1559.294	1628.074	68.780
F ₃		3010.246**	202.169	362.185	882.687	1003.415	120.728	0.879
F ₄		5421.472**	1969.118	836.712	1528.253	2364.966	836.712	0.646
F ₅		3560.731**	94.365	609.623	983.702	1593.326	609.623	0.617
F ₆		8155.633**	1405.698	1530.465	2208.389	3738.855	1530.465	0.590
Morphine (%)		F ₂	3.072**	0.015	0.023	1.016	1.039	0.023
	F ₃	3.016**	0.004	0.047	0.989	1.005	0.015	0.984
	F ₄	2.498**	0.204	0.979	0.506	1.485	0.979	0.340
	F ₅	6.356**	1.565	1.247	1.703	2.950	1.247	0.577
	F ₆	17.831	3.574	11.867	1.988	13.855	11.867	0.143

*P=0.05; *P=0.01

s²_g, Genotypic variances; s²_p, phenotypic variances; s²_e, environmental variances

Table 3 Genetic gain (%) of subsequent generation over base population (F₂) in opium poppy

Crosses	Capsule number/plant				Capsule size (cm ²)				Seed yield (g)			
	F ₃	F ₄	F ₅	F ₆	F ₃	F ₄	F ₅	F ₆	F ₃	F ₄	F ₅	F ₆
'Gz' × 'BR 230'	-10.66	-8.44	-22.58	-7.92	17.36	6.30	1.68	-3.86	-4.15	-8.48	-36.80	-33.10
'NB 1' × 'BR 241'	-13.30	-9.39	-24.74	-9.09	15.65	5.85	0.73	-3.42	-12.45	-10.12	-41.14	-29.95
'ND 5' × 'ND 1002'	-15.15	-8.26	56.26	63.67	8.63	5.06	-14.27	-13.21	-19.31	-19.52	-41.91	-2.05
'NB 5' × 'BR 241'	-13.19	-12.64	-34.72	-29.77	10.87	3.58	8.94	-9.85	-24.24	-12.83	-33.11	-42.55
'MOP 541' × 'BR 241'	-21.68	-11.48	-19.62	-5.87	0.07	2.45	8.76	-11.45	-36.65	-20.77	-56.60	-0.11
'MOP 541' × 'NB 11'	-13.71	-4.52	-6.67	-3.06	10.99	1.55	6.69	3.19	-34.26	-19.18	-54.68	-3.69
'UO 601' × 'BR 231'	-30.42	-14.89	-43.37	-24.56	8.76	9.74	23.03	18.01	-31.97	-14.44	-47.26	1.88
'UO 601' × 'BR 234'	-25.85	-5.72	-3.88	23.98	17.16	7.06	-4.52	-6.85	-23.83	-12.31	-58.58	-1.08
'ND 1002' × 'BR 234'	-36.37	-15.16	-17.45	16.01	14.47	8.22	4.07	-0.14	-8.74	-6.86	-53.42	1.71
'ND 1001' × 'NB 11'	-23.28	-5.46	-4.90	23.51	7.37	4.63	-4.91	-0.29	-29.81	-14.23	-32.27	-2.64
'ND 1001' × 'IS 13'	-27.64	-12.93	-33.75	-23.58	2.68	-0.34	-11.92	-12.80	-44.30	-22.05	-60.84	-0.52
'ND 1001' × 'UO 1285'	-36.37	-15.07	-32.72	-10.80	18.80	9.12	0.99	3.18	-26.05	-12.23	-50.93	-0.63
'BR 226' × 'UO 1285'	-22.26	-11.33	-26.19	-4.82	7.33	0.99	-7.84	-7.45	-30.00	-19.70	-49.85	-3.43
'BR 230' × 'IS 16'	-25.81	-14.54	-32.86	-21.09	5.00	-1.20	-12.98	-12.11	-29.72	-19.57	-55.78	-37.06

Crosses	Opium yield/plant (mg)				Morphine (%)			
	F ₃	F ₄	F ₅	F ₆	F ₃	F ₄	F ₅	F ₆
'Gz' × 'BR 230'	8.05	-7.58	-11.83	-30.64	-10.21	-4.27	-11.03	-3.11
'NB 1' × 'BR 241'	-31.54	-37.40	-38.15	-41.59	-4.02	-3.89	-8.49	-3.90
'ND 5' × 'ND 1002'	-40.36	-25.31	-24.22	-33.80	-35.71	-7.20	-6.26	-17.08
'NB 5' × 'BR 241'	-36.10	-34.33	-34.54	-44.27	-0.74	-3.22	-13.73	-2.75
'MOP 541' × 'BR 241'	-32.43	-49.29	-38.76	-42.09	-31.75	-13.43	-6.54	-7.72
'MOP 541' × 'NB 11'	-37.98	-38.89	-31.30	-33.02	-40.36	1.14	0.73	-3.84
'UO 601' × 'BR 231'	-35.32	-28.92	-31.62	-40.75	-48.98	-11.39	-4.65	-8.23
'UO 601' × 'BR 234'	-30.68	-40.99	-32.38	-36.84	-8.41	-12.22	-6.49	-8.07
'ND 1002' × 'BR 234'	-22.01	-35.12	-35.20	-41.50	-19.02	-5.16	-3.32	3.60
'ND 1001' × 'NB 11'	-11.19	-29.15	-37.35	-24.32	-14.47	-2.63	-1.14	-2.39
'ND 1001' × 'IS 13'	-37.77	-41.63	-37.83	-37.46	-41.76	-6.05	-6.57	-6.22
'ND 1001' × 'UO 1285'	-38.33	-33.03	-26.91	-26.31	-44.71	-4.52	-7.28	-11.11
'BR 226' × 'UO 1285'	-29.27	-26.44	-19.91	-36.34	-30.46	12.63	-0.98	-2.73
'BR 230' × 'IS 16'	-5.21	-37.05	-43.89	-26.96	-6.80	-6.28	-9.81	-2.27

all the crosses (Table 2). The heritability varied from 71.4 to 97.7% in F₂, 60.4 to 98.4% in F₃, 25.8 to 64.6% in F₄, 57.7 to 82.1% in F₅ and 14.3 to 59.0% in F₆ generation which indicated significant genetic variation for yield components among crosses. The knowledge of heritable variability is crucial for proper breeding programme and selection of suitable genotypes in desired direction. Broad sense heritability provides an indication of the importance of genetic variance in relation to total variance. (Goncalves *et al.* 2008). The values of broad sense heritability decreased from F₂ to F₄ for all the traits and suddenly increased in F₅ generation for all the traits and finally decreased in F₆ generation. Low heritability was also reported earlier by Bhandari *et al.* (1997) for seed yield, opium yield and morphine content. The continuous decreasing heritability from F₂ to F₆ generation showed a clearcut trend of increasing homozygosity for all the traits in the crosses. It might be due to selection of desirable plants, followed by selfing of selected plants. The sudden enhancement in heritability for all the

traits in F₅ generation was might be due to highly favourable environmental effects as our present crop opium poppy is highly environment-sensitive crop and can easily be affected by the favourable environmental conditions (Shukla *et al.* 2004). Generally, heritability estimates are influenced by experimental error as it is a part of denominator used in estimation of heritability. The degree of uniformity in test environment influences the experimental error. Moreover, multilocation testing will also provide more reliable estimates of heritability than testing at one location. Hence, heritability estimates in F₅ generation might be validated.

The estimates of predicted genetic gain in different generations for all the traits are shown in Table 3. Genetic advance is the product of heritability and selection differential expressed in terms of unit of standard deviation and thus provides an added edge over heritability in selection programme. In the present study, genetic gain was expressed as a percentage of mean for F₃, F₄, F₅ and F₆ generations over F₂ generation. The results based on overall analysis of

Table 4 Distribution of promising selected crosses in different groups for different traits

Group	Capsules/plant	Capsule size (cm ²)	Seed yield (g)	Opium yield (mg)	Morphine (%)
I		'Gz' × 'BR 230', 'NBRI 1' × 'BR 241', 'UO 601' × 'BR 234', 'ND 1002' × 'BR 234', 'ND 1001' × 'IS 13'	'NBRI-5' × 'BR241', 'Gz' × 'BR230', 'NB-5' × 'ND1002'	'UO 601' × 'BR 231', 'Gz' × 'BR 230', 'NB 1' × 'BR 241', 'NBRI 5' × 'BR 241', 'MOP 541' × 'NBRI 11', 'ND 1002' × 'BR 234', 'BR 230' × 'IS 16'	'ND 1001' × 'NB 11', 'NB 5' × 'R 241', 'ND 1001' × 'UO 1285', 'BR 230' × 'IS 16'
II		'NBRI 5' × 'ND 1002', 'ND 1001' × 'NBRI 11', 'ND 1001' × 'UO 1285', 'BR 226' × 'UO 1285', 'BR 230' × 'IS 16'			'MOP 541' × 'NBRI 11'
III		'MOP 541' × 'BR 241', 'UO 601' × 'BR 231', 'NB 5' × 'BR 241', 'MOP 541' × 'NB 11'			
IV				'ND1001' × 'NBRI 11'	'ND 1001' × 'IS 13'
V	'NBRI 5' × 'BR 241', 'Gz' × 'BR 230', 'NB 1' × 'BR 231', 'MOP 541' × 'BR 241', 'UO 601' × 'BR 231', 'ND 1002' × 'BR 234', 'ND 1001' × 'IS 13', 'ND 1001' × 'UO 1285', 'BR 226' × 'UO 1285', 'BR 230' × 'IS 16'		'MOP 541' × 'NB 11', 'NBRI 1' × 'BR 241', 'UO 601' × 'BR 234', 'ND 1002' × 'BR 234', 'ND 1001' × 'IS 13', 'ND 1001' × 'UO 1285', 'BR 226' × 'IS 16', 'BR 230' × 'IS 16'	'NBRI 5' × 'ND 1002', 'MOP 541' × 'BR 241', 'UO 601' × 'BR 231', 'UO 601' × 'BR 234', 'BR 230' × 'IS 16'	'Gz' × 'BR 230', 'NBRI 1' × 'BR 241', 'NBRI 5' × 'ND 1002', 'MOP 541' × 'BR 241', 'UO 601' × 'BR 231', 'UO 601' × 'BR 234', 'BR 230' × 'IS 16'
VI			'MOP 541' × 'BR 241'		
VII				'ND 1001' × 'IS 13', 'ND 1001' × 'UO 1285'	'ND 1002' × 'BR 234'
VIII	'NBRI 5' × 'ND 1002', 'UO 601' × 'BR 234', 'ND 1001' × 'NBRI 11'				

pattern of genetic gain for each trait showed different trends for different traits. Hence, for convenience to interpret the results to reach on fruitful conclusion, the crosses showing similar pattern over different traits in each generation were grouped accordingly. So, 14 crosses were grouped into 8 distinct groups (Table 4).

The promising selected crosses for different traits were distributed in different groups and presented in Table 4. The promising crosses for different traits in group I showed decline in genetic gain from F₃ to F₆ generation. Similarly, different crosses of group II had increasing pattern of genetic gain in F₃ and decline in F₄ and F₅ and then increase in F₆. Group III showed increasing pattern of genetic gain from F₃ to F₅ generation and finally decreased in F₆ generation, group IV showed decreasing trend in genetic gain up to F₄, followed by increasing trend from F₅ to F₆ generation, group V had crosses having decreased genetic gain from F₃ to F₅ which finally increased in F₆ generation, group VI had single cross showed decline in genetic gain from F₃ to F₆ except in F₅ generation, group VII had declined in genetic gain in F₃ and F₆ generation and increase in F₄ and F₅ and finally the last group VIII showed increasing pattern in genetic gain from F₃ to F₆ generation. The groups I, II, IV, V, VI and VII had crosses which showed reduction in genetic gain from generation to generation due to accumulation of favourable genes and attaining the homozygosity that is governed through dominance gene actions but discrepancies that occurred among the continuous pattern might be due to environmental effects or manual sampling error. Similarly groups III and VIII had crosses which showed increasing pattern in genetic gain from generation to generation and accumulation of favourable alleles. However, some discrepancies in particular patterns might again be due to environmental effects or manual sampling error.

Based on overall analysis, the cross 'MOP541' × 'BR241' for 4 traits and cross 'Gz' × 'BR230' for 3 traits followed similar pattern of genetic gain. These crosses can easily be utilized in composite breeding programme. It was also noticed from the present study that capsule numbers, capsule size, seed yield and opium yield were positively associated with each other and negatively with morphine which suggested that increase in one is directly associated with increase in other, while for morphine it is *vice versa*, as also reported earlier by Yadav *et al.* (2006). Based on this study it is suggested that selection process for isolation of promising lines/crosses would be more powerful if it is based on genetically and phenotypically correlated traits instead of selection based on single trait. Multiple correlated trait breeding seems to be an important tool for enhancing the selection response instead of individual trait selection procedure which can bias the results, as the lines remaining one to be considered for a second trait do not represent a random sample of the entire parental population (Bauer and Leon 2008). In general, seed and opium yield are the most

important traits in selective process of crosses/genotypes and can easily be increased by using multiple correlated trait selection method. Simultaneously, multiple stage testing would be beneficial during selection process to avoid undesirable and inefficient stages of selections. Another very important aspect in selection is that the characters chosen for selection criteria must be least affected by the environment and directly or indirectly affect each other. Anbessa *et al.* (2006) illustrated that knowledge of relative contribution of genetic components and environmental effects in controlling the variation for different traits is important for the successful breeding programme. Opium poppy is a self-pollinated crop having high degree of cross pollination varying from 7 to 40% (Bhandari 1990). Hence, the crop shows inbreeding depression in the subsequent generations after selfing, so to maintain crop vigour sibling (intermating) would be more preferable instead of selfing in the later generations after following effective selection strategies, as suggested earlier by Shukla *et al.* (1999).

REFERENCES

- Ahmed H M, Kandhro M M, Laghari S and Abro S. 2006. Heritability and genetic advance as selection indicators for improvement in cotton (*Gossypium hirsutum* L.). *Journal of Biological Sciences* **6**: 96–99.
- Anbessa Y, Warkentin T, Vandenberg A and Bandara M. 2006. Heritability and predicted gain from selection in components of crop duration in divergent chickpea cross populations. *Euphytica* **152**: 1–8.
- Bauer A M and Leon J. 2008. Multiple-trait breeding values for parental selection, in self-pollinating crops. *Theoretical and Applied Genetics* **116**: 235–42.
- Bhandari M M, Gupta R, Sharma P P and Joshi A. 1997. Path analysis in opium poppy (*Papaver somniferum* L.). *Indian Journal of Genetics and Plant Breeding* **57**: 14–18.
- Bhandari M M. 1990. Outcrossing in opium poppy (*P. somniferum* L.). *Euphytica* **48**: 167–9.
- Ceolin A C G, Goncalves M C V, Vidigal P S F, Kvitschal M V, Gonela and Scapim C A. 2007. Genetic divergence of the common bean (*Phaseolus vulgaris* L.) group Carioca using morpho-agronomic traits by multi-variate analysis. *Hereditas* **144**: 1–9.
- Cruz C D and Carneiro P C S. 2003. Modelos biome ´tricos aplicados ao melhoramento gene ´tico. Vol. 2, 1 Vicosa (MG): UFV, Ed. Imprensa Universitaria.
- Evans L T. 1993. *Crop evolution, Adaptation and Yield*. Cambridge University Press. England.
- Goncalves, V M C, Freddy M, Thai's S B, Roxille E F M and Lara daniel de souza. 2008. Heritability of quantitative traits in segregating common bean families using a Bayesian approach. *Euphytica* **164**: 551–60.
- Pride R R A and Stern E S. 1954. A specific method for determination of morphine. *Journal of Pharmaceutical Pharmacy* **6**: 590–606.
- Shukla S and Singh S P. 1999. Effect of inbreeding in opium poppy (*Papaver somniferum*). *Journal of Agricultural Sciences* **69** (2): 136–9.
- Shukla S and Singh S P. 2004. Exploitation of interspecific

- crosses and its prospects for developing novel plant type in opium poppy pp. 210–39. (*P. somniferum* L.). (in) *Herbal Drugs and Biotechnology*. Trivedi P C (Ed.), Pointer Publishers, Jaipur.
- Shukla S, Yadav H K, Chatterjee A and Singh S P. 2004. Capsule shrinkage in relation to capsule area, opium yield and alkaloid content in *P. somniferum* L. *Physiology and Molecular Biology of Plants* **10**: 253–61.
- Singh S P, Shukla S and Khanna K R. 1995. The opium poppy. (in) *Advances in Horticulture: Medicinal and Aromatic Plants*. Vol. **11** pp 535–74. Chaddha K L and Rajendra Gupta (Eds), Malhotra Publishing House, New Delhi.
- St. Martin S K and Futi X. 2000. Genetic gain in early stages of a soybean breeding program. *Crop Science* **40**: 1559–64.
- Yadav H K, Shukla S and Singh S P. 2006. Genetic variability and interrelationship among opium and its alkaloids in opium poppy (*P. somniferum* L.). *Euphytica* **150**: 207–14.