Electronic Journal of Plant Breeding, 1(4): 649-655 (July 2010)

Research Article

Genetics of morphine, yield and its candidate characters in opium poppy (*Papaver somniferum* L.)

Dinisha Abhishek and Chetan Kumar Choudhary

Abstract
Combining ability in opium poppy, *Papaver somniferum* L. was analyzed through full diallel cross comprising five parents, 20 F_{1s} (including reciprocals) and 20 F_{2s} (including reciprocals). The analysis of variance revealed significant differences for all the characters demonstrating considerable variation among genotypes. Estimates of components of general and specific combining ability variances exhibited equal importance of both additive and non additive gene actions for the expression of all the characters. However, higher magnitude of SCA components of variance indicated preponderance of non –additive effects for all the traits except for leaves/plant, seed yield and opium yield in F_2 generation. The average degree of dominance was more than unity, showed over dominance and also conferred above findings. Among the parents NB-1KR401-3/3 for capsules/plant, stem diameter, capsule wt/plant, capsule size, peduncle length and seed yield, NB-5KR3-2-2/1 for days to 50% flowering, plant height leaves/plant, capsule wt/plant, and seed yield, Papline and 58/1 for plant height and opium yield and NB-5KR40-7/2/-3 for morphine content were found good general combiners. Inclusion of lines with good GCA in a single or multiple crosses followed by intermating i.e. population improvement approaches may be expected to offer genetic improvement in breeding for higher opium, seed yield and its component traits.

Key words: General combining ability, specific combining ability, inbred lines, *Papaver somniferum, degree of dominance*

Introduction

The opium poppy (*Papaver somniferum* L.) is an important medicinal plant of immense pharmaceutical uses. India is world's largest producer of opium. Besides meeting domestic demand, India exports opium and its derivatives of worth Rs. 13 million worldwide (Singh et al. 1995; Shukla and Singh 2004). In recent years, global demand for specific alkaloids especially for thebaine, codeine and morphine is increasing drastically which require an urgent need to develop high latex yielding varieties rich in specific alkaloids to maintain India's position in world market. Such varieties can be conveniently developed through breeding approaches. The successful breeding program depends mainly on a judicious selection of promising parents from gene pool, a clear cut understanding of genetic mechanism involved in the inheritance of characters,which help the breeders in deciding the most appropriate breeding procedure to enhance the genetic potentialities.It is also desirable that selection of suitable parents for hybridization should be based on the combining ability of a particular line to nick well with other lines and produce superior promises. So, to identify potentially superior parents and hybrids, information on the combining ability is needed which would also be helpful to determine the pattern of gene effects in the expression of quantitative traits.

The general combining ability (GCA) of each parent should be examined with the objective to develop superior genotypes while specific combining ability (SCA) provides information about the performance of hybrids (Cruz and Regai 1994). The differences in the GCA are mainly due to the additive genetic effects and higher order additive interactions, while the differences in SCA are attributed to the non-additive dominance and other types of epistasis (Falconer1989). This analysis allows broad inferences on the nature of gene effects for a trait under selection. Based on this information, breeder

Division of Plant breeding, college of Agriculture, C.C.S. Haryana Agriculture University, Hisar
Email: dinisha82@gmail.com

can make suitable strategy to select desirable parents or can also determine which breeding procedure will effciently improve the performance of the traits of interest (Dudley and Moll 1969). Several mating designs namely diallel, partial diallel, line × tester, biparental mating and triple testcross have been used in different crops by different workers to characterize nature and magnitude of gene effects. However, these models have their limitations due to certain postulations.Among several mating designs,the diallel cross analysis (Griffing 1956 b) is an efficient evaluation device,though it involved limited number of parents, is only biometrical tool, which not only reduces the testing period but also gives all the useful genetic information on the basis of which coherent breeding plan be chalked out in early generations. Previous studies showed that the variation in opium latex, seed yield and its component traits and major alkaloids content was controlled by genes acting additively and non additively. Singh et al. (2001) reported non-additive gene action for plant height, capsule length, days to maturity, husk yield/plant, seed yield/plant and morphine content. Lal and Sharma (1991) reported additive component for morphine and codeine content. Shukla and Khanna (1997) reported additive gene action for plant height and capsule/plant and nonadditive for days to maturity, opium yield/plant, seed yield/plant and dry weight of plant while both additive and non-additive genetic variances were important for stem diameter, days to 50 %flowering, capsule size and morphine content. The information already available on various genetic parameters from one set of material could not be applied to other. It was therefore essential to evaluate some more lines for their breeding value and understanding other genetic parameters related to yield and is component traits and alkaloid contents.With these considerations, the present investigation in opium poppy was under taken to study the genetics general combining ability and mode of gene action for various important traits.

Material and methods

The experimental material for the present study comprises of 5 pure and diverse genotypes selected from the germplasm line maintained by selfing at the National Botanical Research Institute (NBRI),Lucknow, India (Singh et al, 1995, 1996).These genotypes were crossed in a full diallel fashion (Griffing 1956) to obtain 20 F_1.The trial comprising 20 F_1s, 20 F_2s and 5 parents was conducted during crop year 2006-2007 at the experimental field of National Botanical Research Institute (NBRI), Lucknow located at $26^0$4'N latitude and $80^0$45'E longitude and altitude of 129 m above sea level. All the entries were evaluated in a randomized block design with three replications. Two rows of each entry were grown in each replication with a plant-to-plant spacing 10 cm and row to row 30 cm.The experiment was bordered by planting two rows around it to minimize border effects. Standard cultural practices were followed throughout the crop season. Ten competitive plants in each test entry per replication were tagged before flowering and observations were recorded on days to 50% flowering, plant height (cm), leaves/plant, branches/plant, capsules/plant, capsule size (cm^2), capsule weight/plant(g), seed yield/plant(g), husk yield/plant(g) and opium yield/plant(mg)

HPLC analysis

The opium latex of four successive lancing from the capsule of tagged plants at the interval of 3-4 days was collected and air-dried. The dried opium latex was powdered and subjected to quantification for major alkaloid morphine, through HPLC following the method suggested by Khanna and Shukla (1986). The chromatographic analysis was done by using waters (Milford, USA). High pressure liquid chromatography consisting of M6000A solvent delivery system, 717 plus auto sampler, µ Bondapak C18 column (4 mm i.d.× 250 mm) 996 PDA detector and millennium 320software.

Statistical analysis

The mean data were subjected to statistical analysis following the method suggested by Griffing (1956) to obtain the combining ability variances and gene actions using the software windostat,Hyderabad,India.

Results and discussion

The study of the nature and magnitude of the gene action governing various characters is essential for formulating efficient breeding program for increasing productivity. In the present study, analysis of variances showed significant differences among parents and hybrids suggesting the presence of genetic diversity among them.

Combining ability variances and gene actions

Combining ability variances were estimated to ascertain the nature and magnitude of generations involved in the inheritance of different characters for F_1 hybrids and F_2 families. These estimates were translated into genetic variances due to additive and non-additive components. The additive gene action is largely due to the results of additive genetic variance while non-additive is due to dominance and epistatic type of gene action. The analysis of variance for GCA and SCA were highly significant for all the characters indicated that parents and crosses differ significantly in both the generations (Table 1). The significant differences of GCA and SCA variances exhibited equal importance of additive and non-additive gene actions for all the characters. The estimates of component of variance (Table 1) due to δ^2 g and due to δ^2s also indicated both additive and non-additive gene actions for the expression of all the characters under study. However, the higher magnitude of δ^2s than δ^2g indicated preponderance of non-additive gene action for days to 50% flowering, plant height, peduncle length, capsules/plant, seed yield/plant, capsule size, capsule weight/plant, husk yield/plant, morphine content and opium yield/plant in both the generations and leaves/plant, in F_2 generation. The ratio of mean square component associated with variance of GCA and SCA (δ^2g/δ^2s) was much more than the theoretical maximum of unity for all the traits except leaves/plant in F_1.These results tend to suggest that genetic variation among crosses was primarily of non-additive type.

The average degree of dominance more than unity showed over dominance, which confirmed the above findings. Kandalkar et al. (1992) and Singh et al.(1996, 2001) have also reported non-additive genetic variance for capsules/plant, capsule weight/plant, leaves/plant, opium yield/plant and seed yield/plant.However, additive genetic variance for days to 50% flowering, plant height, leaves/plant, capsule diameter,capsules/plant, capsule weight/plant, latex yield, seed yield/plant, husk yield/plant, morphine, codeine,narcotine and straw morphine, was reported by various workers (Lal and Sharma 1991; Shukla 1992; Kandalkar et al. 1992; Kandalkar and Nigam 1993; Shukla et al. 1993; Singh et al. 2002, 2003). The discrepancies in the nature of gene action reported by different workers might be due to differences in parental diversity in the material, size of the population, design adopted and environmental conditions in which the experiment wasconducted. In addition to other genetic parameters, the degree of dominance is also of interest to plant breeders (Gardner 1963). In the present investigation, all the traits showed over-dominance except leaves/plant in F_1 generation, where partial dominance was operating. This observed over dominance at gene level may be spurious since particular combination of positive and negative alleles or a complementary type of gene action or simply correlated gene distribution, may seriously inflate the mean degree of dominance and convert partial dominance into apparent over-dominance (Hayman 1954). The opium poppy is self-pollinated crop with varied degree of out crossing (Bhandari 1990), which neither followed model of complete random mating nor those of complete inbreeding, instead the mating systems of the instant population is partial inbreeding (Patra et al. 1992). Thus breeding systems of both self and cross-pollinated crops are utilized in poppy (Singh et al. 1995; Shukla and Singh 1999). The higher portion of non-additive genetic variance for most of the traits indicated that it is desirable and important to maintain heterozygosity in the population for the improvement purposes. Since non-additive genetic variability is not fixable, the breeding methods such as bi-parental mating followed by recurrent selection may play a greater role in genetic improvement of a crop (Singh and Singh 1987).

General combining ability effects

A basic requirement in any effective hybridization program is to identify superior genotypes which could excel in their combining ability. General combining ability effects plays a major role in making choice of parents and also isolation of germplasm base for utilization in hybridization program for further improvement. In the present study, none of parent was found as good general combiner for all the traits (Table 2). Among the parents the best general combiners in the both the generations were NB-1KR401-3/3 for capsules/plant, capsule wt/plant, peduncle length and seed yield, NB-5KR3-2-2/1 for days to 50% flowering, plant height, leaves/plant, and capsule wt/plant, Papline and 58/1 for plant height and opium yield and NB-5KR40-7/2-3 for morphine content.

The GCA effects include additive and additive × additive components of gene action (Griffing 1956; Sprague 1966) which represents fixable genetic variance. In view of this, breeders may utilize the good general combiners in specific breeding program for improvement of yield and its component traits(Yadav et al.2009). It appears that the GCA rank for yield is related to the GCA for the important component traits. Thus the parents NB-1KR401-3/3, NB-5KR3-2-2/1, Papline and 58/1 could be utilized extensively in hybridization followed by selection to accelerate the pace of genetic improvement of yield and alkaloid content. It concluded that in order to synthesize a dynamic population with most of the favorable genes accumulated, it will be pertinent to make use of these parents, which are good general combiner for various characters, in a multiple crossing program or an intermating population involving all possible crosses among them subjected to bi-parental mating to supplement speedy recombination and also breaks genetic barrier, if present (Jensen 1970).

References

Bhandari, M.M. 1990. Outcrossing in opium poppy (*P. somniferum* L.). Euphytica 48:167–169. doi:10.1007/BF00037196

Cruz, C.D, and A.J. Regai. 1994. Modelos biometricos applicados ao melhoramento genetic. Universidade Federal de Vicosa, Imprensa Universitara. Vicosa, Minas Gerais, Brazil

Dudley JW, Moll RH (1969) Interpretation and use of estimates of heritability and genetic variance in plant introduction. Crop Sci 9:257–262

Falconer, D.S. 1989. Introduction of quantitative genetics, 3rd edn. Longaman, Essex, pp 275–276

Gardner, C.O.1963. Estimation of genetic parameters in cross fertilizing plants and their implication in plant breeding. Statistical genetics and plant breeding. NAS-NRS Publ 982:225–252

Griffng, B. 1956. Concept of general and specific combining ability in relation to diallel crossing system. Aust J Biol Sci 9:463–493

Hayman, B.I. 1954. The theory and analysis of diallel crosses. Genetics 39:789–809

Jensen, N.F. 1970. A diallel selective mating system for cereal breeding. Crop Sci 10:629–635

Kandalkar, V.S. and K.B . Nigam. 1993. Combining ability for physiological characters and opium yield in opium poppy (*Papaver somniferum* L.). Indian J Genet 53:34–39

Kandalkar, V.S., H, Patidar and K.B. Nigam. 1992. Combining ability analysis for harvest index, seed yield and important component characters in opium poppy (*Papaver somniferum* L.). Indian J Genet 52:275–279

Khanna, K.R. and S. Shukla. 1986. HPLC investigation of the inheritance of major opium alkaloids. Planta Med 54:157–158. Doi:10.1055/s-2007-969106

Lal, R.K. and J.R. Sharma. 1991. Genetics of alkaloids in *Papaver sominferum*. Planta Med 57:271–274. doi:10.1055/s-2006-960088

Patra, N.K., R.S. Ram, S.P. Chauhan and A.K. Singh. 1992. Quantitative studies on the mating system of opium poppy (*P. Somniferum* L.). Theor Appl Genet 84:299–302. doi:10.1007/ BF00229486

Shukla, S., K.R. Khanna and S.P. Singh. 1993. Genetic architecture of narcotine in opium poppy *(Papaver somniferym* L.). Indian J Plant Genet Resour 7:139–142

Shukla, S. 1992. Genetics of seed yield and its contributing traits in opium poppy (*Papaver somniferum* L.). Proc Nat Acad Sci India 62((B)II):213–217

Shukla, S. and K.R. Khanna. 1997. Genetic architecture of opium yield, seed yield and its components in opium poppy (*P. somniferum* L.). Advances in Plant Science Research (Ed. Dhir), vol 5 & 6. Interational Book Distributors,Dehradun, pp 43–55

Shukla, S. and S.P. Singh. 1999. Genetic systems involved in inheritance of papaverine in opium poppy (*Papaver Somniferum* L.). Indian J Agric Sci 69:44–47

Shukla, S. and S.P. Singh. 2004. Exploitation of interspecific crosses and its prospects for developing novel plant type in opium poppy (*P. somniferum* L.). In: Trivedi PC (ed) Herbal drugs and biotechnology. Pointer Publishers, Jaipur, pp 210–239

Singh, H.P., R.K. Tewari, S.P. Singh, A.K. Singh and N.K. Patra. 2002. Genetic studies in opium poppy (*P. somniferum* L.). J Med Arom Plant Sci 24:762–765

Singh, S.P. and H.N. Singh. 1987. Combining ability in relation to year interaction in okra. *Abelmoshus esculentus* (L.) Moench. SABRAO J 19:93–101

Singh, S.P., H.P. Singh, A.K. Singh, and R.K. Verma. 2001. Identification of parents and hybrids through line × tester analysis in opium poppy (*Papaver somniferum*). J Med Arom Plant Sci 22(23):327–330

Singh, S.P., S. Shukla and K.R. Khanna. 1995. The opium poppy. In:Chaddha KL, Gupta R (eds) Advances in horticulture:medicinal and aromatic plants, vol 11. Malhotra Publishing house, New Delhi, pp 535–574

Singh, S.P., S. Shukla and K.R. Khanna. 1996. Diallel analysis for seed yield and its components in opium poppy (*P. somniferum*). J Med Arom Plant Sci 18:259–263

Singh, S.P., S. Shukla and A. Chatterjee 2003. Studies on diVerent selection parameters in opium poppy (*P. somniferum* L.). J Med Arom. Plant Sci 25:8–12

Sprague,G .F. 1966. Quantitative genetics in plant improvement. In Plant Breeding (Ed. Kenneth, J Frey). A symposium on Plant Breed. Iowa State Univ Press Ames, Iowa, pp 315-354

Yadav, H.K., S. Shukla, and S.P. Singh. 2009. Genetic combining ability estimates in the F_1 and F2 generations for yield, its component traits and alkaloids content in opium poppy(*Papaver somniferum* L.)

Table 1 Analysis of variance showing mean square for combining ability in opium poppy (*Papaver somniferum* L.) in F_1 and F_2 generations.

Source		Hybrid	GCA	SCA	GCA/SCA	Error	$\delta_2 g$	$\delta_2 s$	$\delta_2 g/ \delta_2 s$	$(\delta_2 s / \delta_2 g)^{1/2}$
Days to 50%	F_1	21.99**	69.87**	8.99**	7.77	0.50	2.38	2.83	0.84	1.09
Flowering	F_2	21.91**	50.69**	14.09**	3.59	1.12	1.43	4.32	0.33	1.74
Plant height	F_1	150.38 **	427.97**	75.04**	5.70	12.73	13.79	20.77	0.66	1.23
	F_2	97.86 **	145.45**	84.94**	1.71	17.16	2.36	22.59	0.10	3.09
Leaves/plant	F_1	10.61**	35.38**	3.88**	9.11	0.81	1.23	1.02	1.20	0.91
	F_2	6.48 **	18.47**	3.22**	5.73	0.82	0.59	0.80	0.74	1.16
Peduncle length	F_1	4.18**	10.45**	1.64**	6.37	0.41	0.58	0.79	0.73	1.36
	F_2	2.12**	8.26**	2.59**	3.18	0.48	0.46	0.68	0.67	1.47
Capsules/plant	F_1	0.62**	1.45**	0.39**	3.72	0.15	0.04	0.08	0.52	1.39
	F_2	0.78 **	0.89**	0.75**	1.18	0.12	0.01	0.21	0.03	6.46
Capsule size	F_1	4.03**	12.27**	1.79 **	6.85	0.44	0.40	0.45	0.91	1.05
	F_2	2.96 **	4.18**	2.62**	1.59	0.32	0.06	0.77	0.08	3.55
Capsule	F_1	13.83**	29.14**	9.67**	3.01	1.08	0.76	2.86	0.27	1.94
weight/plant	F_2	6.95**	14.78**	4.82**	3.06	1.16	0.38	1.22	0.32	1.77
Seed	F_1	5.93**	9.98 **	4.83**	2.06	0.59	0.20	1.42	0.14	2.65
yield/plant	F_2	2.79**	4.38**	2.36**	1.85	0.70	0.07	0.55	0.15	2.64
Husk	F_1	2.94**	7.38**	1.73**	4.26	0.40	0.22	0.44	0.50	1.41
yield/plant	F_2	1.53**	3.58**	0.97**	3.67	0.46	0.10	0.17	0.59	1.29
Opium	F_1	6093**	15187**	3624**	4.18	263	452.0	1120	0.40	1.57
yield/plant	F_2	3284 **	7203**	2220**	3.24	204	194.8	672	0.28	1.86
Morphine	F_1	9.84**	25.29**	5.64**	4.48	0.59	0.76	1.68	0.45	1.48
	F_2	10.26**	26.98**	5.72**	4.72	0.48	0.83	1.74	0.47	1.45

Table 2 Estimates of GCA effects for 5 parents of full diallel cross in respect of yield its candidate traits and morphine content in opium poppy (*Papaver somniferum* L.)

Parents	Days to 50% flowering		Plant height		Peduncle length		Leaves/plant		Capsule/plant		Stem diameter	
	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2
Papline	1.540**	-0.333	1.420	2.517*	-1.205*	-0.178	1.374*	-0.169	-0.264*	-0.08	-0.018	-0.010
NB-5KR40-7/2-3	0.073	-0.333	-1.729	1.052*	0.251	1.018*	-0.748	-0.037	-0.254*	-0.001	-0.071*	-0.046
NB-1KR401-3/3	0.107	-1.133**	-3.24	0.495	1.283**	.650*	-2.438**	1.110	0.403*	0.366*	0.021	0.030
NB-5KR3-2-2/1	-2.493**	-0.867*	2.911*	1.134*	1.090*	-0.312	0.818*	1.864*	0.202	-0.050	0.064*	0.076*
58/1	-0.277	1.733**	-4.699**	-2.199*	1.296**	-0.128	0.195	-2.768*	0.214	-0.008	0.05	-0.083*
S.E.(gi)	0.188	0.146	0.833	0.766	0.253	0.192	0.239	0.586	0.077	0.069	0.019	0.021
C.D. at 5%	0.596	0.463	2.649	0.849	0.805	0.609	0.761	1.863	0.244	0.223	0.062	0.065
C.D. at 1%	1.093	0.849	4.864	4.473	1.277	1.119	1.397	3.419	0448	0.408	0.113	0.118

Contd..

Parents	Capsule size (cm^2)		Capsule weight		Seed yield/plant		Husk yield/plant		Opium yield/ plant		Morphine %	
	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2
Papline	-0.630*	-0.471	-1.837**	-0.905*	-0.400	-0.811*	-0.445*	-0.084	6.885**	6.253**	-1.480*	-0.113
NB-5KR40-7/2-3	-1.125**	0.738	-0.450	-0.132	-0.505	-0.131	0.074	0.016	-4.768**	-14.859**	1.423*	1.434*
NB-1KR401-3/3	0.119	0.58	2.165**	0.807*	1.225**	1.208**	0.081	0.994**	0.299	0.102	-0.164	-0.366
NB-5KR3-2-2/1	0.698*	-0.336	0.880*	0.708*	0.501	0.158	0.341	-0.046	-7.835**	-12.452**	-1.345	-0.612
58/1	0.046	-0.138	0.100	-1.255*	0.150	-0.425	-0.051	-0.880**	5.419**	43.666**	0.332	0.034
S.E.(gi)	0.139	0.269	0.223	0.241	0.182	0.184	0.114	0.148	0.851	0.451	0.723	0.728
C.D. at 5%	0.445	0.854	0.766	0.709	0.585	0.577	0.362	0.469	2.708	1.436	1.417	1.426
C.D. at 1%	0.817	1.567	1.406	1.301	1.059	1.074	0.664	0.861	4.971	2.635	1.858	1.870

*,**Significant at 5 and 1% respectively