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Genetic Analysis of Capsule and Its Associated Economic Traits in Opium Poppy (*Papaver somniferum* L.)

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Four single crosses (VG20 × SGE48, SGE48 × SG35II, VG26 × SG35II, and SG35II × VG20) in opium poppy (*Papaver somniferum* L.) were analyzed to study the gene actions involved in the inheritance of quantitative traits, namely plant height, branches/plant, capsules/plant, peduncle length, capsule index, stigmatic rays, straw yield/plant, and morphine content. Simple additive, dominance, and epistatic genetic components were found to be significant for inheritance pattern. Dominance effect (*h*) was higher than additive effect (*d*). Digenic interaction indicated the prevalence of dominance × dominance (*l*) followed by additive × dominance (*j*) type epistasis. The significance of dominance (*h*) and dominance × dominance (*l*) indicated duplicate epistasis for all the traits and crosses except SG35II × VG20 for stigmatic rays. Biparental mating followed by recurrent selection involving desired recombinants may be utilized to improve the component traits.

Key words: *capsule, epistasis, gene effects, joint scaling test, morphine content, straw yield*

Opium poppy (*Papaver somniferum* L.) is an important medicinal plant known to produce more than 80 alkaloids belonging mainly to phenanthrene and tetrahydrobenzylisoquinoline groups (Weid et al. 2004). Pharmaceutically important alkaloids include the analgesic morphine, codeine, antispasmodic papaverine, etc. (Facchini and Park 2003). These alkaloids are harvested from mature capsules and straw of the fully grown plant. Information on the nature and magnitude of gene effects is required for genetic improvement of any crop plant. In opium poppy, such genetic information has been obtained from several studies, mainly through either of the 2 mating designs, that is, diallel (Lal and Sharma 1991; Kandalkar et al. 1992; Singh et al. 1996, 2004; Yadav et al. 2007, 2009; Kumar et al. 2008;) or line × tester (Singh et al. 2001) designs. The diallel mating designs have some unrealistic assumptions and limitations of handling fewer numbers of parental lines at a time, and the line × tester design mainly adopted for preliminary testing of large number of general combiners. Cavalli (1952) proposed a joint scaling test for testing the expected relationship between generation means based on the additive–dominance model of Mather and Jinks (1971). This test has 2 important edges over other first-degree statistics methods: 1) the generation means are not, in general, known with equal precision, and hence, appropriate weights are given to them and their expectations; 2) the data used in this analysis are neither confined to single generation nor restricted in any other way. Furthermore, the analysis is less cumbersome in respect to calculations, errors are smaller, and the estimates are reliable. The generation mean and its partitioning provides the information on the type of gene action involved. The study on this aspect is, however, meager in opium poppy under concentrated poppy straw system, where the morphine is extracted from straw. Thus, the present investigation was undertaken to understand the particular gene action involved in the inheritance of capsule and its associated characters contributing to straw morphine.

Materials and Methods

The material consisted of 6 generations, P_1, P_2, F_1, F_2, B_1, and B_2, of 4 crosses involving 4 parental lines, SG35II, SGE48, VG20, and VG26 (Table 1), their 4 single crosses (VG20 × SGE48, SGE48 × SG35II, VG26 × SG35II, and SG35II × VG20) along with their respective 4 F_2s and 8 backcrosses [B_1's ($F_1 \times P_1$) and B_2's ($F_1 \times P_2$)]. Thus, a total of 24 treatments (4 crosses and their 6 basic generations, P_1, P_2, F_1 ($P_1 \times P_2$), F_2 (selfed F_1's), B_1 ($F_1 \times P_1$), and B_2 ($F_1 \times P_2$), respectively) were raised in a randomized block design with 3 replications at the Central Institute of Medicinal and Aromatic Plants, Lucknow, India, during 2 consecutive years 2003–2004 and 2004–2005.

The planting was done in 3-m long rows with row-to-row and plant-to-plant distances of 30 and 10 cm, respectively. Each generation was represented by 3 rows (as experimental

Table 1 Salient features of the parental lines of opium poppy

S. no.	Name	Features	Origin
1	SG35II	Broad leaves, large capsule size, high seed yielder, low morphine content in straw, tall, and white petal color.	It is a mutant strain derived from variety Sanchita on irradiation with γ-rays (15 kR). (Satpute 2000; Kumar 2007).
2	SGE48	Broad and less serrated (compared with Sanchita) leaves, medium capsule size, high seed yielder, low morphine content in straw, tall, and white petal color.	This mutant strain derived from variety Sanchita on treatment of combined mutagen (5 kR γ-rays + 0.4% EMS). (Satpute 2000; Kumar 2007).
3	VG20	Broad leaves, medium capsule size, early flowering, high morphine content in straw, tall, and white petal color.	This an induced mutant derived from variety Vivek on irradiation with γ-rays (15 kR). (Satpute 2000; Kumar 2007).
4	VG26	Broad leaves, large capsule size nonwaxy apsule surface (telia), high seed yielder, medium morphine content in straw, tall, and white petal color.	This is a mutant derived from variety Vivek upon irradiation with γ-rays (15 kR). (Satpute 2000; Kumar 2007).

rows) and 2 rows as nonexperimental rows grown as the border rows in each replication in order to minimize competition of nutritional uptake. Five competitive plants per treatment/replication were randomly selected and observations were recorded on plant height (cm), number of branches and capsules/plant (number), peduncle length (cm), capsule index (capsule width/capsule length), number of stigmatic rays (notches) on main capsule, straw yield/ plant (gm), and morphine content (%). The morphine content in straw was quantified through high performance liquid chromatography analysis (Akhila and Uniyal 1983). Data were processed on HCL Infiniti Pro computer (P4) using the software SPAR-1 (Doshi and Gupta 1991).

Statistical analysis was performed separately for each cross. A Waller Duncan *K* ratio was applied to determine the significant differences that existed among the generation means. The interacting and noninteracting crosses were identified following the methods of A, B, C, and D scales ($A = 2B_1 - P_1 - F_1 = 0$; $B = 2B_2 - P_2 - F_1 = 0$; $C = 4F_2 - 2F_1 - P_1 - P_2 = 0$; $D = 2F_2 - B_1 - B_2 = 0$) suggested by Hayman and Mather (1955). The observed means of the 6 generations were used to estimate "*m*" (a constant), "*d*" (pooled additive effects), and "*h*" (pooled dominance effects) as per the joint scaling test of Cavalli (1952). The adequacy of additive–dominance model was tested by comparing the observed and expected means and goodness-of-fit tested against the χ^2 value for 3 degrees of freedom (df) (the number of observed means available minus the number of parameters to be estimated). The 3 parameters—*m*, *d*, and *h*—were estimated from the 6 generations by weighted least squares using reciprocals of the squared standard errors of each mean weight.

Results and Discussion

The results obtained from the joint scaling test analysis are shown in Electronic Supplementary Material (ESM) Table 2 (noninteracting crosses) and ESM Table 3 (interacting crosses) that have been elaborated and discussed here under the heads of noninteracting crosses and interacting crosses. The basis for identification of the noninteracting crosses and interacting crosses was the test of scales A, B, C, and D.

Noninteracting Crosses

Among the noninteracting crosses, the cross VG20 × SGE48 topped the list (for 5 traits, i.e., branches/plant, capsules/ plant, peduncle length, stigmatic rays, and capsule index) and was followed by SG35II × VG20 (branches/plant, capsules/ plant, peduncle length, and straw yield/plant). The other 2 noninteracting crosses were SGE48 × SG35II and VG26 × SG35II each for 2 traits (i.e., plant height and stigmatic rays and branches/plant and capsules/plant), respectively. Because the χ^2 (3 df) values were nonsignificant, the additive–dominance model was found to be adequate for these traits and crosses. Furthermore, both additive and dominance components were significant for plant height in the cross SGE48 × SG35II and for peduncle length in VG20 × SGE48, whereas dominance was observed in crosses VG26 × SG35II (branches/plant and capsules/plant) and SG35II × VG20 (peduncle length) and additive in cross VG20 × SGE48 (capsules/plant). Nonsignificance of both the components, that is, additive and dominance for crosses VG20 × SGE48 (branches/plant, stigmatic rays, and capsule index), SG35II × VG20 (branches/plant, capsules/plant and straw yield/plant), and SGE48 × SG35II (stigmatic rays) may be ascribed to the 2 facts: 1) either the estimates are very low or their standard errors are very high and 2) near to symmetrical distribution of positive and negative alleles among the parents and hence leading to the intercancellation of the effect of each other.

Interacting Crosses

Interacting crosses too were identified through the scaling tests, and a further confirmation was made through the adequacy test (χ^2) and the 6-parameter model was applied (ESM Table 3). The opposite signs for *h* and *l* indicated the predominance of duplicate (D) type of epistasis and their similar signs indicated complementary (C) type of epistasis. The observed findings on estimates of various genetic components have been briefly described below.

Plant Height

Under Indian conditions, shorter plant type is desirable to avoid the lodging. More number of branches bear more number of capsules, longer peduncle length is desirable because it contributes to straw yield used for straw alkaloid production, higher capsule index is desirable for maximum seed and straw yield, higher number of stigmatic rays is desirable for maximum number of carpel as well as larger surface area for maximum pollination and higher straw yield and morphine content. Among the 4 crosses, only 3 (VG20 × SGE48, VG26 × SG35II, and SG35II × VG20) showed the best fit of 6-parameter model (ESM Table 3). The crosses VG20 × SGE48 and SG35II × VG20 exhibited significant positive estimates for "*d*" (additive) component indicating the additive type of inheritance of plant height. This implied that inheritance of plant height was controlled by associated additive gene pair, as noted earlier (Narain et al. 2007). Two crosses, VG20 × SGE48 and VG26 × SG35II, exhibited significant negative estimate for "*h*" (dominance) component, suggesting that the genes with negative effects were dominant over the genes with positive effects (Pawar et al. 1988; Kumar 2007).

Among the interallelic interaction, components "*i*" (additive × additive) and "*j*" (additive × dominance) were found negatively significant in all the 3 crosses, whereas "*l*" (dominance × dominance) was positively significant in crosses VG20 × SGE48 and VG26 × SG35II. In the cross VG20 × SGE48, the magnitude of "*l*" was larger than "*d*" for plant height that may impose restrictions on progress through selection (Tefera and Peat 1997; Kumar 2007).

Branches/Plant and Capsules/Plant

For branches and capsules/plant, only one cross SGE48 × SG35II exhibited best fit of the 6-parameter model (ESM Table 3). The significantly positive estimates for "*h*" and "*l*" component indicated the role of associated gene pairs in the inheritance of branches and capsule/plant (Narain et al. 2007).

Peduncle Length

The crosses SGE48 × SG35II and VG26 × SG35II fitted best the 6-parameter model for peduncle length (ESM Table 3). All the components, that is, "*d*," "$h^{(-\text{ve})}$," "$i^{(-\text{ve})}$," "$j^{(-\text{ve})}$," and "*l*" registered significant estimates (signs indicated in parenthesis against them) for the cross VG26 × SG35II, whereas only "*l*" component registered significant negative estimate for the cross SGE48 × SG35II. Simultaneous significance of "*d*" and "*h*" with opposite signs and larger magnitude of "*h*" may impose hindrances in selections as pointed out earlier (Tefera and Peat 1997).

Capsule Index

Out of 4 crosses, only 3 (SGE48 × SG35II, VG26 × SG35II, and SG35II × VG20) showed the best fit of 6-parameter model for capsule index (ESM Table 3). The components "*h*," "*i*," and "*l*" registered significant estimates irrespective of their signs in all the 3 interacting crosses, whereas "*d*" with smaller magnitude was significant for crosses SGE48 × SG35II and SG35II × VG20 and "*j*" for crosses SGE48 × SG35II and VG26 × SG35II. In all the 3 crosses, the magnitude of "*h*" and "*l*" was larger than the "*d*" irrespective of their signs implying the major role of duplicate epistasis. This suggested that the selection for this trait might be difficult in these crosses due to restricted variability (Tefera and Peat 1997; Kumar 2007).

Stigmatic Rays (Notches) of Main Capsule

Of the 2 interacting crosses VG26 × SG35II and SG35II × VG20 for this particular trait, only VG26 × SG35II showed the best fit to 6-parameter model for stigmatic rays (ESM Table 3). Higher estimates of "*h*" and "*l*" than "*d*" for this cross exhibited the importance of dominant gene effect for this trait, as observed by some researchers (Narain et al. 2007). The nonsignificant estimates of "*d*," "*h*," "*i*," "*j*," and "*l*" for the cross SG35II × VG20 suggest that trigenic or higher order of interactions are required for understanding the inheritance of this trait.

Straw Yield/Plant

Among the 3 interacting crosses VG20 × SGE48, SGE48 × SG35II, and VG26 × SG35II, only VG20 × SGE48 and SGE48 × SG35II showed the best fit to the 6-parameter model, whereas the cross VG26 × SG35II required higher order interaction (trigenic or more) model for straw yield/plant (ESM Table 3). Significant estimates of "*i*" for SGE48 × SG35II implied the importance of additive component for this trait (Kumar 2007). The significant estimate of "*j*" component for the cross VG20 × SGE48 may be ascribed to intercancellation of positive and negative effects of the alleles.

Morphine Content

All the 4 interacting crosses showed the best fit to the 6-parameter model (ESM Table 3). Significant estimates of "*d*" for all the crosses irrespective of the sign exhibited that inheritance of morphine content was controlled by additive gene action. The occurrence of significant but negative estimates for "*h*" component in the cross SGE48 × SG35II suggested that the genes with negative effects were dominant over the genes with positive effects, as shown earlier (Pawar et al. 1988; Kumar 2007). However, Shukla et al. (1994) and Shukla and Singh (1999) reported the role of both additive (*d*) and dominance (*h*) in control of morphine with prevalence of dominance effect based on additive–dominance model. For interallelic interaction, the cross SG35II × VG20 exhibited significant positive estimates of "*i*" and "*j*" components. For "*j*" component, the 2 crosses (VG20 × SGE48 and SGE48 × SG35II) showed significant negative estimates. For "*l*" component, the cross SGE48 × SG35II exhibited significant positive estimate.

Overall, the gene effects analysis in the present study have highlighted the greater existence of dominance (*h*) effects and dominance × dominance (*l*) nonallelic interactions for the characters studied. However, the opposite sign (+ − and − +) of dominance (*h*) and dominance × dominance (*l*) indicated the presence of duplicate (D) epistasis for all the characters and crosses except SG35II × VG20 for stigmatic rays. The presence of duplicate epistasis may reduce the variability in F_2 and further generations, thereby reducing the progress of selection. It was also evident from this study that in opium poppy breeding strategies for overall improvement would not be feasible and that it should be trait oriented and parent selection must be done with utmost care. Under these circumstances, the use of intermating of the best parents followed by recurrent selection holds promise for genetic improvement of these traits in opium poppy.

Supplementary Material

Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

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