# GGE Biplot Analysis Based on Diallel for Exploitation of Hybrid Vigour in Opium Poppy (*Papaver somniferum* L.)

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## ABSTRACT

The diallel cross design is frequently utilized to obtain information on genetic effects, estimates of General and Specific Combining Ability (GCA and SCA) and to identify promising heterotic combinations as well as heterotic patterns. In the present study, heterotic crosses were identified for specific alkaloids in opium poppy (*Papaver somniferum* L.) following Yan's GGE Biplot model by use of 5×5 full diallel data. The results obtained through biplot analysis were compared with those obtained through Griffing's to check and confirm the accuracy of Yan's GGE biplot model. Parents A (papline), B (NB5KR40-7/2-3), and E (58/1) were identified as good general combiners. The crosses B×C, B×E and E×B for morphine, C×D and C×E for narcotine, and A×B, A×C and A×E were identified as heterotic combinations. None of the crosses were found heterotic for codeine and thebaine.

**Keywords:** Alkaloids, Biplot, Diallel, GCA, Heterosis, SCA.

## INTRODUCTION

In recent years the global demand for opium alkaloids and its derivatives are steadily increasing in the pharmaceutical industries (Shukla and Singh, 2004). India is one of the largest producers of licit opium, a source of about 80 valuable alkaloids. Among them the demand for five major opium alkaloids namely: morphine, codeine, thebaine, narcotine, and papaverine has increased multifold (Shukla and Singh, 2004; Shukla *et al.,* 2006)**.** In general the varieties/cultivars of the species *Papaver somniferum* grown under cultivation, contain less thebaine and codeine (1-2%). Hence, efforts are being made worldwide to explore the possibilities for the enhancement of these specific alkaloids. The scientific breeding approach for the improvement of *P. somniferum* for increased alkaloid content, have been going on in India. A considerable genetic variability has been noticed for major alkaloids within the

indigenous germplasm of this species, extensively utilized in various hybridization programmes (Yadav *et al.,* 2006, 2007). The opium poppy is a photosensitive crop, the temperate germplasm lines of which cannot be easily hybridized because of non-synchronous flowering on the one hand and the distinct cytological variation (which harbor the incorporation of new favorable genes in the cultivated poppy species) on the other hand (Shukla and Singh, 2004). As for hybrid breeding programs, evaluation requires a determination of both combining ability and *per se* performance of the parents. Abel and Pollak (1991) suggested that the effective value of a germplasm depends on its heterotic response to other genotypes. Thoughtful utilization of local germplasm/accessions and the search for alternative heterotic patterns are therefore important research objectives. To achieve the targeted goal, several breeding techniques are extensively utilized. Among such techniques diallel mating design is

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frequently utilized to obtain information on genetic effects, estimates of General (GCA) and Specific (SCA) Combining Ability, heritability for a population of parental lines and to identify the promising heterotic combinations as well as heterotic patterns (Bertoria *et al.,* 2006). In the conventional diallel approach, GCA is only related to an evaluation of parents whereas SCA is associated with the crosses. Conventional diallel is too less interpretative and designed only for describing the phenotypic performances of the crosses. Thus, to overcome the lacuna of the conventional diallel approach, recently Yan (1999), and Yan and Hunt (2002) have proposed a model identified as GGE Biplot to analyze the diallel data based on biplot constructed from the first two principal components (PC1 and PC2) derived from PC analysis of environment centered yield data. The merits of biplot over conventional diallel are its graphical presentation which enhances the capability to understand the data as more interpretative, and trying to interpret the phenotypic variation of the crosses. Yan *et al.* (2001) demonstrated through empirical evidences that entry PC1 scores had near perfect correlation with entry main effects (GCA effects) if the latter are higher than 35% of total GGE variation; otherwise the variation explained by PC1 would represent the main effect. Because the PCs are least square solutions, PC1 alone explains at least as much variation as the one

typically more than that of GCA.

To trap the increasing global demand of opium alkaloids, out of the few lines rich in alkaloid content developed through various breeding approaches, five stable high alkaloid containing lines were crossed in all the possible combinations in diallel fashion to enhance the specific alkaloids with the objectives of: (i) to determine the genetic potential of the selected lines as source of major alkaloids and to identify the heterotic groups among populations; (ii) to compare findings of both Griffing and biplot analysis by using diallel data as well as to check the accuracy of results obtained through GGE software results following the removal of reciprocal differences in identifying heterotic combination.

## MATERIALS AND METHODS

Five pure lines namely Papline or Papaverine line (Parent A), 58/1 (Parent E), NB5KR40-7/2-3 (Parent B), NB1KR40-3/3 (Parent C), NB530+2-2/1 (Parent D) were hybridized in full diallel crosses in all possible combinations including reciprocals to get the $F_1$ seeds (Table 1). A field trial comprised of 5 parents and 20 $F_1$s was conducted in a Randomized Block Design (RBD) with three replications in year 2006-07 at the experimental field of National Botanical Research Institute, Lucknow, Uttar Pradesh, India, situated between $26^0$N

**Table 1**. Pedigree/Origin information related to parents employed in hybridization.

| S. No | Parents | Pedigree/Origin |
|---|---|---|
| 1. | Papline (A) | Spontaneous Mutant selected from variety Jawahar Aphim and after selection for several years, developed as a distinct variety carrying high papaverine content. |
| 2. | NB5KR40-7/2-3(B) | Line developed from stable high yielding variety NBRI-5 as a result of mutation breeding through gamma radiation and followed by selection upto three generations bearing high thebaine content. |
| 3. | NB1KR40-3/3 (C) | Line developed from stable high yielding variety NBRI-1 as a result of mutation breeding using gamma radiation followed by selection upto two generations carrying high thebaine content. |
| 4. | NB530+2-2/1 (D) | Line developed from stable high yielding variety NBRI-5 as a result of mutation breeding through gamma radiation followed by selection upto three generations continually carrying high thebaine content. |
| 5. | 58/1 (E) | Selection from germplasm no. BR316 possessing high thebaine content. |

and 80.5$^0$E, while being 120 m above sea level. Each entry was grown in two rows per replication with row to row spacing of 30 cm and plant to plant distance of 10 cm. The rows were 3m in length. Recommended cultural practices were applied (Yadav *et al.,* 2006). The opium was individually collected from randomly selected ten competitive individual plants from each replication of F$_1$s and of parents through repeated four lancings within the interval of 4-5 days. The opium was dried and weighted to calculate the yield per plant (mg). The dried and powdered opium was subjected for the alkaloids (morphine, codeine, thebaine, narcotine and papaverine) estimation through HPLC (Khanna and Shukla, 1986). The mean values per replication for each estimated alkaloid were made use of for statistical analysis as based on model proposed by Griffing (1956) (method 1) and GGE biplot statistical model (Beta version) proposed by Yan (1999), and Yan and Hunt (2002).

The results were presented in two ways: (i) according to Yan and Hunt (2002) i.e. the Average Tester Coordinate (ATC) view and (ii) Polygon view. In the ATC view the results were displayed according to the average tester position which is a virtual tester whose PC1 and PC2 scores are equal to the average PC1 and PC2 scores across all testers. The ATC was established with its abscissa passing through the origin and perpendicular to the abscissa. Thus the GCA effects of the entries are approximated by their projections onto the ATC abscissa. The parallel lines perpendicular to the abscissa help in ranking the entries in terms of GCA. The projections of the entries onto ATC coordinate display their SCA effects which represent the tendency of the entries to produce superior hybrids with specific testers. The polygon view of the biplot provides a best way of graphical presentation for visualizing the interaction pattern between entries and testers. It is drawn by connecting the entries. The perpendicular line to each side drawn from the origin of the plot divides the biplot into

several sectors with each tester falling into one sector. Tester falling into the same sector shares the same best mating partner, which is the entry at the vertex of the polygon in that sector. Tester that falls in different sectors is of different best mating partners. Entries located near the biplot origins are less responsive to the change of the testers. Contrasting groups of entries relative to the ATA may be regarded as different heterotic groups. As a rule in Biplot analysis for the diallel data, each genotype was considered as an entry and a tester. In the present study, significant differences were observed among F$_1$ generations. So, that data can not be considered as both an entry and a tester. To overcome this situation, two types of data sheets were prepared to present biplot analysis i.e. one data sheet with both side straight crosses data and the other with both side reciprocal crossing data. The polygons were drawn by using same data as entry and tester for both straight and reciprocal crosses and were utilized to identify the heterotic combinations of direct and reciprocal crosses respectively. A third type of data entry sheet was also prepared using both straight and reciprocal crosses as an entry and a tester respectively with biplot being drawn after reciprocal differences being removed through the Yan's GGE software (2001) to check the accuracy in identifying the heterotic combinations.

## RESULTS

The analysis of variance based on diallel analysis showed pronounced significant differences among the genotypes. Hybrids as well as reciprocals were highly significant for all the traits. Parent vs F$_1$s were also highly significant for all the traits except for morphine while reciprocals *vs.* parents were significant for thebaine and narcotine (Table 2).

The results as based on Griffing model showed that GCA and SCA variances were recorded as highly significant for all the

**Table 2.** Analysis of variance, variances and estimates of combining ability for some alkaloids in Opium Poppy (*Papaver somniferum*).

| Source of variation | Morphine | Codeine | Thebaine | Narcotine | Papaverine |
|---|---|---|---|---|---|
| Replication (df 2) | 1.79 | 0.06 | 0.05 | 0.17 | 0.05 |
| Treatment (df 24) | 10.04** | 2.15** | 5.35** | 14.79** | 1.77** |
| Parents (df 4) | 13.80** | 1.77** | 4.31** | 6.12** | 0.22* |
| $F_1$s (df 9) | 7.58** | 0.54** | 3.19** | 26.43** | 2.49** |
| Reciprocals $F_1$s (df 9) | 12.88** | 0.77** | 4.25** | 6.78** | 1.70** |
| Parents *vs.* $F_1$s (df1) | 1.60 | 32.41** | 41.89** | 25.91** | 2.92** |
| Reciprocal vs. parents(df 1) | 0.04 | 0.01 | 2.22** | 5.52** | 1.01 |
| Combining ability variances | | | | | |
| GCA | 5.64** | 1.13** | 3.24** | 8.84** | 1.79 |
| SCA | 5.164** | 1.17** | 2.12** | 4.99** | 0.56 |
| Reciprocals | 0.62** | 0.09** | 0.86** | 3.30** | 0.14 |
| GCA/SCA | 1.09 | 0.97 | 1.53 | 1.77 | 3.22 |
| Error | 0.17 | 0.02 | 0.01 | 0.02 | 0.02 |
| Combining ability estimates | | | | | |
| $\delta^2 g$ | 0.07 | 0.00 | 0.12 | 0.41 | 0.13 |
| $\delta^2 s$ | 2.98 | 0.69 | 1.26 | 2.96 | 0.32 |
| $\delta^2 e$ | 0.17 | 0.02 | 0.01 | 0.02 | 0.02 |
| $\delta^2 g/ \delta^2 s$ | 0.02 | 0.00 | 0.10 | 0.14 | 0.40 |
| $(\delta^2 s/^2 g)^{0.5}$ | 6.47 | 18.53 | 3.20 | 2.69 | 1.59 |

\*, \*\* Significant at 5 and 1% respectively.

traits in $F_1$ generation except for papaverine (Table 2). Parent A was found to be best general combiner followed by B for morphine, parent E followed by B for codeine, parent B followed by parents E and D for thebaine, parent E followed by parents C and D for narcotine and while parent A for papaverine content (Table 3). Highest SCA effect in straight crosses was observed in cross B×E followed by crosses D×E, B×C and A×C and as well for cross D×A in the reciprocal crosses for morphine. Similarly, no cross as for straight crosses and only one cross D×C for reciprocal for codeine while crosses B×E and A×B among straight crosses and crosses C×B, B×A plus D×C for reciprocals for thebaine revealed the highest SCA effects. The crosses C×E followed by B×D and C×D for straight crosses, and crosses E×C followed by D×C in reciprocal crosses for narcotine while cross A×C followed by A×E and A×D in straight crosses and no cross in reciprocals for papaverine showed the highest SCA effect (Table 3). Heterosis was studied over the better parent. The highest heterotic

combinations in straight crosses were B×E, B×C and C×E as well as E×B, E×C and E×D in reciprocal crosses for morphine, crosses C×D and C×E in straight crosses while none in reciprocal cross for narcotine and crosses A×B, A×C, A ×D and A x E in straight crosses and crosses B×A, C×A, D×A and E×A in reciprocals for papaverine showed heterotic combinations (Table 4). However, no heterotic combination was noticed for codeine and thebaine.

The biplot of straight crosses explained 81.5% (Figure 1-a) and 82.9% for the reciprocals (Figure 1-b) for morphine, 65.2% for straight (Figure 2-a) and 69.3% (Figure 2-b) for reciprocals regarding codeine, 85.8% for straight (Figure 3-a) and 86.5% (3-b) for reciprocals as regards thebaine, 85.8% for straight (Figure 4-a) and 86.5% (Figure 4-b) for reciprocals for narcotine as well as 97.6% for straight (Figure 5-a) and 94.9% (Figure 5-b) for reciprocal of papaverine in the total variations respectively. Based on the projection onto the ATC abscissa, B and A showed highest GCA in both the straight and

**Table 3.** Estimates of GCA and SCA values for some alkaloids in opium poppy (*Papaver somniferum*).

| Parents | Morphine | | Codeine | | Thebaine | | Narcotine | | Papaverine | |
|---|---|---|---|---|---|---|---|---|---|---|
| Papline (A) | 0.79 ** | | -0.48 ** | | -0.82 ** | | -0.52 ** | | 0.73 ** | |
| NB5KR40-7/2-3(B) | 0.73 ** | | 0.12 *** | | 0.64 ** | | -1.30 ** | | -0.01 | |
| NB1KR40-3/3 (C) | -0.25 * | | -0.12 ** | | -0.30 ** | | 0.46 ** | | -0.13 ** | |
| NB530+2-2/1 (D) | -0.97 ** | | 0.04 | | 0.19** | | 0.23** | | -0.24 ** | |
| 58/1 (E) | -0.30* | | 0.44** | | 0.30** | | 1.14** | | -0.34** | |
| S.E. | 0.11 | | 0.04 | | 0.03 | | 0.04 | | 0.04 | |

| Crosses | SCA | REC$^a$ | SCA | REC | SCA | REC | SCA | REC | SCA | REC |
|---|---|---|---|---|---|---|---|---|---|---|
| A×B | 0.15 | 0.51 | -0.35** | 0.01 | 0.27** | 0.52** | -2.22** | -1.01** | 0.16 | -0.08 |
| A×C | 0.70* | 0.26 | -0.06 | 0.02 | -0.45** | -0.88** | -0.82** | -0.74** | 0.88** | -0.05 |
| A×D | -0.33 | 0.85** | -0.54** | 0.01 | -0.85** | -0.48** | -0.92** | -0.72** | 0.22* | 0.03 |
| A×E | -1.77** | -0.07 | -0.10 | -0.47** | -0.56** | -0.06 | -0.31** | -0.85** | 0.46** | 0.22 |
| B×C | 1.17** | -0.94* | -0.50** | 0.07 | -1.03** | 0.87** | -0.91** | 0.01 | 0.00 | -0.60** |
| B×D | -2.55** | 0.59 | -0.54** | 0.01 | -1.23** | -0.02 | 1.18** | -0.54** | -0.04 | -0.44** |
| B×E | 2.43** | -0.66* | -0.19* | -0.06 | 0.40** | -0.90** | -0.57** | -1.17** | -0.10 | -0.27* |
| C×D | -0.34 | -0.01 | -0.18** | 0.50** | -0.06 | 0.46** | 0.52** | 0.34* | -0.30** | -0.07 |
| C×E | 0.02 | -0.46 | -0.51** | 0.01 | -0.19* | -0.40** | 1.65** | 3.12** | -0.26** | 0.01 |
| D×E | 1.24** | -0.34 | -0.28** | -0.01 | -0.01 | -1.01** | -0.54** | -1.45** | -0.02 | -0.02 |
| SE | 0.23 | 0.28 | 0.07 | 0.08 | 0.06 | 0.07 | 0.08 | 0.10 | 0.08 | 0.10 |
| CD at 5% | 0.54 | 0.66 | 0.16 | 0.20 | 0.15 | 0.18 | 0.19 | 0.24 | 0.19 | 0.23 |
| CD at 1% | 0.79 | 0.97 | 0.24 | 0.29 | 0.22 | 0.26 | 0.28 | 0.34 | 0.28 | 0.34 |

$^a$ Reciprocal, *, ** Significant at 5 and 1% respectively.

**Table 4.** Estimates of heterosis (%) for some alkaloids in Opium Poppy (*Papaver somniferum*).

| Crosses | Morphine | | Codeine | | Thebaine | | Narcotine | | Papaverine | |
|---|---|---|---|---|---|---|---|---|---|---|
| | MP | BP | MP | BP | MP | BP | MP | BP | MP | BP |
| A x B | 4.51* | -4.15 | -40.50** | -50.82** | -17.56** | -37.58** | -75.47** | -79.37** | 165.56** | 124.10* |
| A x C | 8.52** | -8.49** | -32.71** | -40.08** | -81.97** | -84.46** | -38.32 | -46.49* | 586.56** | 360.80** |
| A x D | -7.677** | -15.91** | -46.24** | -54.86** | -73.35** | -79.44** | -40.98** | -49.64* | 236.82** | 215.77** |
| A x E | -11.57** | -26.73** | -39.69** | -50.74** | -44.72** | -51.54** | -32.38 | -36.24 | 667.80** | 284.45** |
| B x A | -2.69 | -10.75** | -41.16** | -51.36** | -40.47** | -54.93** | -52.34* | -59.93** | 193.68** | 147.73** |
| B x C | 13.46** | 3.52 | -40.57** | -45.36** | -35.76** | -45.04** | -26.19** | -28.95 | -100 | -100 |
| B x D | -18.05** | -18.65** | -43.26** | -44.29** | -53.76** | -54.96** | -6.69** | -8.51 | -100** | -100 |
| B x E | 28.53** | 14.91** | -31.87** | -32.89** | -29.48** | -40.61** | -35.15** | -42.73 | -100 | -100 |
| C x A | 4.56 | -11.83** | -1.72 | -41.03** | -33.71** | -42.88** | -21.98** | -32.32 | 621.38** | 384.16** |
| C x B | 29.29** | 17.96** | -44.01** | -48.53** | -69.84** | -74.19** | -26.19** | -28.93 | 185.63* | 75.99 |
| C x D | -4.79 | -12.61** | -24.63** | -29.47** | -31.30** | -39.94** | 16.21** | 13.99** | -100 | -100 |
| C x E | 12.32** | 9.92** | -36.60** | -42.51** | -39.69** | -40.67** | 60.27** | 46.56* | -100 | -100 |
| D x A | -19.60** | -26.77** | -0.57 | -55.04** | -51.27** | -62.45** | -24.89** | -35.92 | 221.06** | 200.99* |
| D x B | -27.18** | -27.72** | -43.63** | -44.66** | -52.84** | -54.07** | 8.39** | 6.29 | -18.93 | 29.63 |
| D x C | -4.67 | -12.51** | -47.08** | -50.47** | -49.93** | -56.23** | 7.08** | 5.04 | -51.11 | -66.24 |
| D x E | 7.45** | -3.30 | -32.67** | -34.86** | -46.67** | -54.40** | -21.09** | -29.12** | -49.76 | -75.04 |
| E x A | 1.27 | -25.78** | -16.82** | -32.05** | -41.26** | -48.51** | -15.40** | -20.23** | 480.57** | 190.70* |
| E x B | 39.93** | 25.10** | -29.23** | -30.29** | 6.423* | -10.37** | -6.55** | -17.46** | 60.10 | -20.15 |
| E x C | 21.07** | 18.49** | -36.58** | -42.49** | -20.35** | -21.63** | -13.38** | -20.79** | -100 | -100 |
| E x D | 13.36** | 2.02 | -32.53** | -34.73** | -4.80 | -18.61** | 13.73** | 2.16 | -22.93 | -61.71 |
| SE | 0.307 | 0.355 | 0.1505 | 0.174 | 2.662 | 3.074 | 0.179 | 0.208 | 0.315 | 0.363 |
| CD 5% | 0.618 | 0.7138 | 0.303 | 0.404 | 5.353 | 6.182 | 0.362 | 0.418 | 0.633 | 0.731 |
| CD 1% | 0.825 | 0.952 | 0.349 | 0.467 | 7.142 | 8.247 | 0.483 | 0.557 | 0.844 | 0.975 |

*, ** Significant at 5 and 1% respectively.
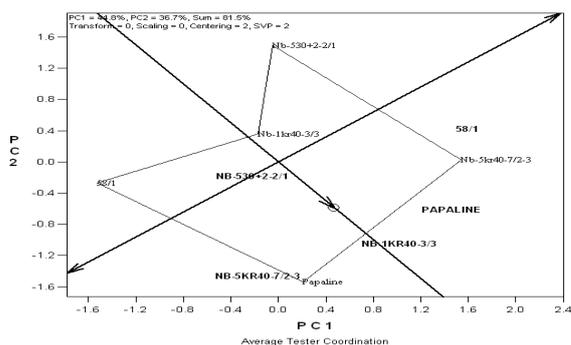
(a)
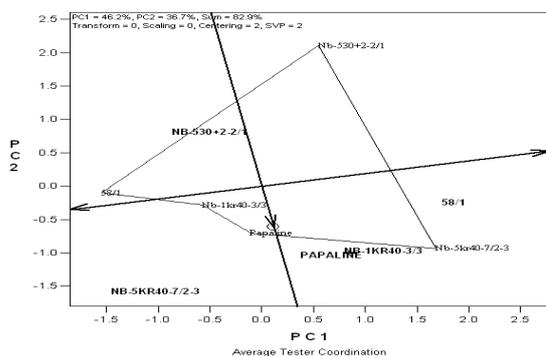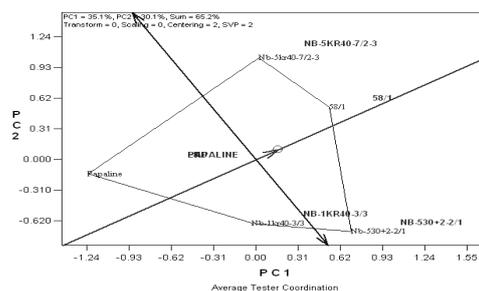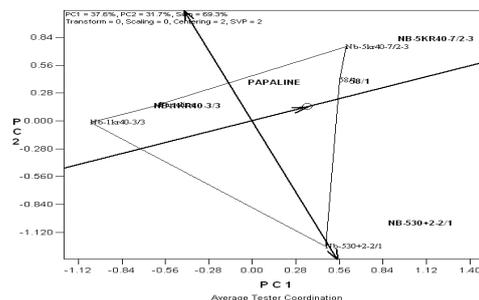


(b)
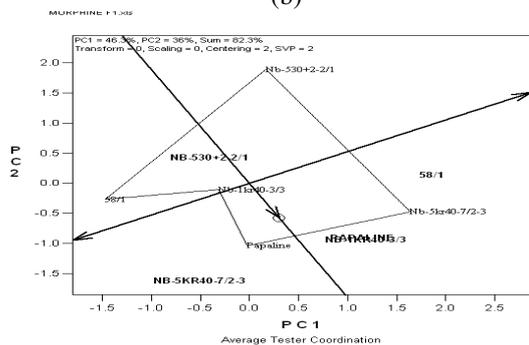


(c)

**Figure 1**. Polygon view of Biplot for morphine: (a) Straight F$_1$; (b) Reciprocal F$_1$, (c) Removing reciprocal differences F$_1$.
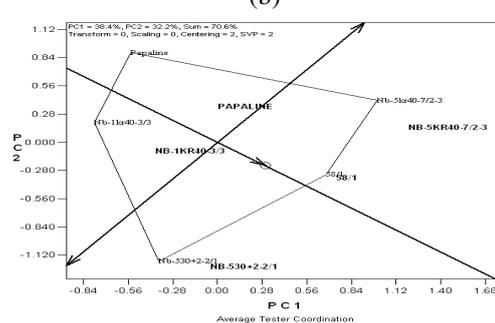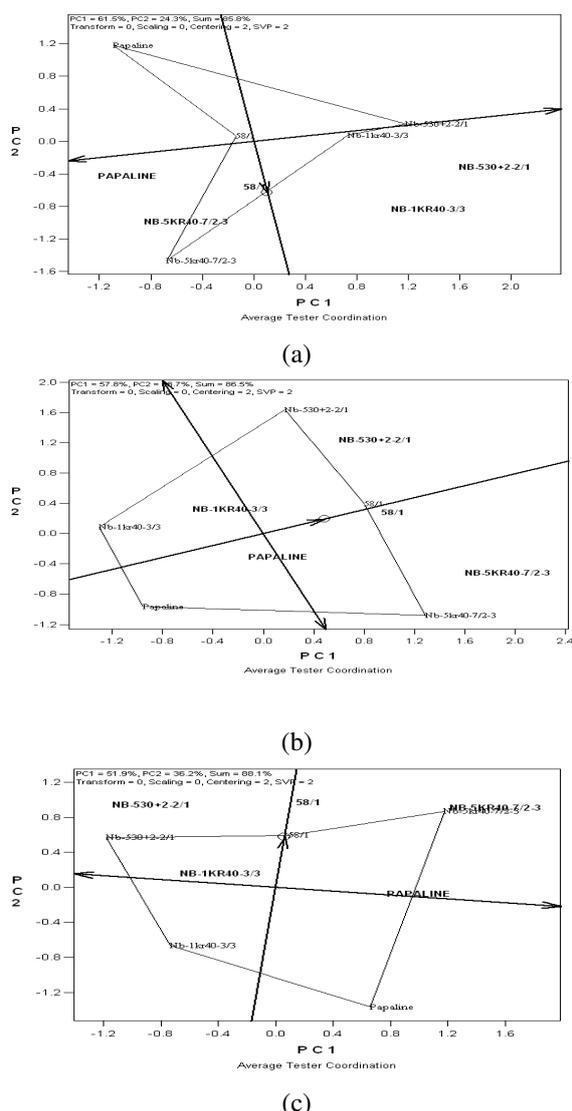


(a)



(b)



(c)
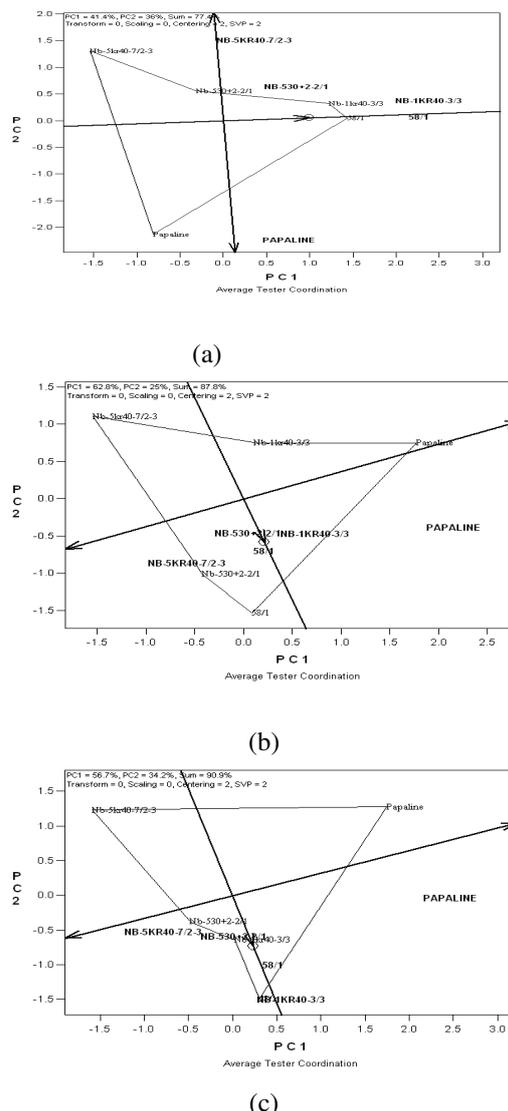
**Figure 2**. Polygon view of biplot for codeine: (a) Straight F$_1$; (b) Reciprocal F$_1$, (c) Removing reciprocal differences F$_1$.

reciprocal crosses (Figures 1-a and 1-b) for morphine, entry D and E bore the highest GCA followed by *B= C> A* for the straight and *B= E= D> A> C* for the reciprocal crosses (Figures 2-a and 2-b) for codeine. B carried the highest GCA followed by *C> D> E> A* for the straight and *B= E> D> A= C* (fall near the ATC) for reciprocal crosses (Figures 3-a and 3-b) for thebaine, C had the

highest GCA followed by *D> A> B* and E (fall near the ATC) for the straight and as well *E> D> A> C> B* for reciprocal crosses (Figures 4-a and 4-b) for narcotine A carried the highest GCA followed by *E> D> C= B* for the straight and *A> B> D> C= E* for the reciprocal crosses (Figures 5-a and 5-b) for papaverine. The ranking of genotypes for all the traits obtained through biplot in general
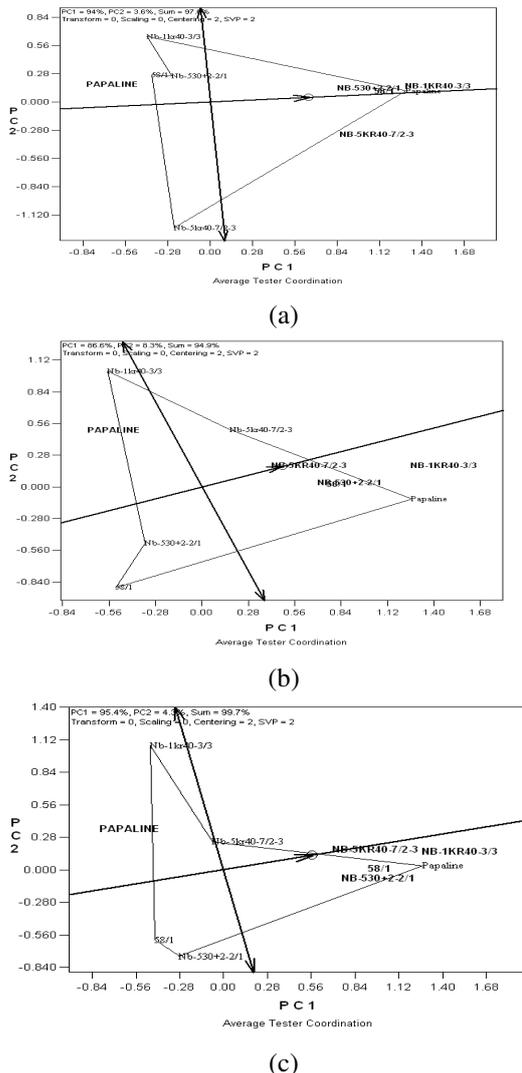
(a)



(b)



(c)

**Figure 3.** Polygon view of biplot for thebaine: (a) Straight $F_1$; (b) Reciprocal $F_1$, (c) Removing reciprocal differences $F_1$.



(a)



(b)



(c)

**Figure 4.** Polygon view of biplot for narcotine: (a) Straight $F_1$; (b) Reciprocal $F_1$, (c) Removing reciprocal differences $F_1$.

supported the findings obtained through Griffing model. Based on the largest projections onto the ATC abscissa the highest SCA was observed for parents B and D in straight crosses and for parents D and B in reciprocals for morphine, entries B and A and C and D for both straight and reciprocal crosses respectively for codeine, entries D and A for straight crosses along with D and C for reciprocals for thebaine, entries D and A for straight crosses plus D and C for

reciprocal crosses for narcotine while entries B, C and D in both straight and reciprocal crosses for papaverine bore the highest SCA effects. The two heterotic groups (C, B, D) and (A, E) for both straight and reciprocals for morphine, (B, A) and (C, D, E) for both straight and reciprocals for codeine, (C, D) and (A, B, E) and (B, A) as well as (E, D, C) for straight crosses and reciprocal for thebaine, (C, B, D) and (A, E) and (C, A) and (E, D, B) for straight and reciprocal

(a)



(b)



(c)

**Figure 5.** Polygon view of biplot for papaverine: (a) Straight F₁; (b) Reciprocal F₁, (c) Removing reciprocal differences F₁.

crosses for narcotine and (A, E, D, C) and (B) and (C, B) and (A, D, E) for straight and reciprocal crosses respectively for papaverine were noticed, suggesting that the hybrids obtained by hybridization between these genotypes would show heterosis which can be verified through the polygon data. The crosses B×E, B×C and B×A were heterotic in sector B in straight crosses for morphine (Figure 1-a) while crosses E×C, E×B and E×D showed heterotic effect in reciprocal as c, d and b fell as testers in sector E for morphine (Figure 1-b), no cross for straight and reciprocal for codeine (Figures 2-a and 2-b) and thebaine

(Figures 3-a and 3-b), one cross D×B for straight crosses (Figure 4-a) and no cross for reciprocal (Figure 4-b) for narcotine and crosses A×B, A×C, A ×D and A×E were heterotic for straight crosses due to the presence of testers b, c, d and e in sector A, one heterotic combination C×D in sector C due to parent D present as entry (Figure 5-a) and crosses E×A, D×A and C×A in sector A, C×A and C×B in sector C and E×D in sector E in reciprocal (Figure 5-b) for papaverine were heterotic.

The biplot analysis excluding reciprocal effects explained 82.3% (46.3 and 36% by PC1 and PC2 respectively) for morphine (Figure 1-c), 70.6% (38.4 and 32.2% by PC1 and PC2, respectively) for codeine (Figure 2-c), 88.1% (51.9 and 36.2% by PC1 and PC2, respectively) for thebaine (Figure 3-c), 90.9% (56.7 and 34.2% by PC1 and PC2 respectively) for narcotine (Figure 4-c) and 99.7% (95.4 and 4.3% by PC1 and PC2, respectively) of the total variation (Figure 5-c) for papaverine respectively. The highest GCA effect was noticed for parent B followed by parent A, E, D and C for morphine, A and B followed by *D> A= C* for codeine, B followed by *D> C> A> E* for thebaine, parent E followed by parent *A= B> C= D* for narcotine and while parent A bore the highest GCA followed by *B> D> C= E* for papaverine. Entry B and D carry the highest SCA for morphine, A and C for codeine and thebaine, A and B for narcotine and C, E and D for papaverine. The two heterotic groups (B, D) and (A, C, E), (E, B, A) and (C, D), (E, B, A) and (C, D), (A) and (B, C, D, E) and (B, C) and (A, D, E) were pinpointed for morphine, codeine, thebaine, narcotine and papaverine respectively. This suggested that hybrids would possibly be expected to be able to show heterosis. Crosses B×E, B×A and B×C were heterotic in sector B, cross E×D in sector E and A×B in sector A for morphine. No crosses for codeine, thebaine and narcotine were observed as heterotic. The crosses A×E, A×C, A×B and A×D in sector A and C×A, B×A and C×B in sector C for papaverine were heterotic.

## DISCUSSION

The selection of suitable combiners (parents) from the material under study and their heterotic crosses in diallel crossing programme are mostly being done following Griffing approach (Griffing, 1956). However, in recent years, Yan and Hunt (2002) provided a quick evaluation method based on the biplot analysis to determine the most suitable combiners and the best heterotic crosses through diallel data. Throughout the present study, diallel data obtained from full diallel set of five parents were subjected to biplot analysis to find out the best heterotic combinations for each trait under study. Parallel to biplot analysis, Griffing's approach to identify the heterotic crosses was also considered to compare the accuracy as well as the authenticity of the biplot results.

The analysis of variance showed widespread significant differences among the parents and $F_1$s which suggested a great level of genetic variability to exist among the parents for all the traits. In the current study the reciprocal differences were also significant which might be due to the presence of maternal effects among the parents for each trait (Durrant, 1965; Shukla, 1985). Singh (1966) based upon his study on Maize, suggested that the source of genotypes, nature of cytoplasm and their inheritance could have a significant bearing on the expression of a particular character. Similarly, the role of maternal effect in the inheritance of different traits has also been reported by Owolade *et al.* (2006) in *Cassava* genotypes and Dhliwayo *et al.* (2005) in maize.

Significant mean square values of GCA and SCA variances suggested the role of both additive and non additive gene actions. The traits morphine, thebaine, narcotine and papaverine carried the higher ratios of $(\delta^2 GCA)/(\delta^2 SCA)$ which indicated predominance of additive gene action in the inheritance of these traits. Similar findings have also been reported by Singh *et al.* (2002, 2003). For codeine, the ratio of $(\delta^2 GCA)/(\delta^2 SCA)$ was less than the one indicating the role of non-additive gene action. In the present study, none of the parents was found to present a proper combiner over all the traits. The parents E, B and A were good combiners for most of the traits as based upon the overall performance of GCA analysis from both Griffing

and biplot analysis. The improvement in opium poppy for specific alkaloids can be easily achieved through hybridization of these parents followed by selection. A composite of proper combiners followed by successive cycles of selection is likely to give high returns in breeding. So, for long term approach "diallel selective mating" can be recommended (Jensen, 1970). A wide range of variation was present within the SCA. However, none of the hybrids had consistently high SCA effects for all the traits. The crosses which showed high SCA and involved both of the parents as suitable combiners, could have sizeable additive×additive gene action which could be fixable in nature (Verma and Srivastava, 2004). In $F_2$ generation these crosses might give transgressive segregants that could be exploited through recurrent breeding and biparental mating. The crosses revealed high×low GCA combinations besides expressing favorable additive effects of the high parent, showing complimentary gene effects and possessing higher SCA effects. The crosses D×C were identified as good specific combiners for three traits *viz.* codeine, thebaine and narcotine, cross B×E for morphine and thebaine as well as cross A×C for morphine and papaverine. However, the crosses bore low×low or average×average general combiners but had high SCA and were generally heterotic due to the interaction between favorable genes contributed by the relevant parents (Verma and Srivastava, 2004). Such a behavior has been attributed to overdominance and epistasis (Satyanarayana *et al.,* 2000). Most of the heterosis displayed by such type of interactions is non- fixable due to the additive×dominance or dominance×dominance type of gene actions which could be further exploited through heterotsis breeding. None of the crosses was found heterotic for all the traits. The crosses B×C, B×E, C×E and E×B for morphine, C×D and C×E for narcotine and the crosses A×B, A×C, A×D and C×A for papaverine were identified as heterotic crosses. It is pertinent to be stated that the hybrids which were much superior over their parents, had parents more genetically different than the others (Hallauer and Miranda, 1981). No cross was found heterotic for codeine and thebaine, though the selected lines/parents undertaken for the study had high thebaine but unable to produce heterotic combinations. It

seemed interesting that only the parents having similar recessive and dominant alleles can exhibit either little or no heterosis (Davenport, 1908). The entries in polygon present in the same group showed heterotic combinations which might be either due to different dominant genes or due to masking effects as in the case of crosses E×D, E×A and B×D for narcotine and A×C, A×D, A×E for straight crosses as well as E×A and D×A for reciprocal crosses for papaverine, while if the entries present in different heterotic groups did not show any heterotic combinations might be due to recessive gene effects.

The study evidently proved the authenticity, ease and importance of Yan's model for analyzing diallel data in comparison with Griffing's. The findings clearly demonstrated that analysis done through GGE biplot software by removing the reciprocal differences could not produce correct information about heterotic crosses if both straight and reciprocal crosses have heterotic effects which biased the identification of promising hybrids due to averaging of both the data. So based on the present study, it is recommended that if the diallel data have significant reciprocal differences, biplot analysis should be done separately instead of using facility of removing reciprocal differences through the software. Some discrepancies that persist among the results analyzed by both the models do not affect the authenticity of biplot model and it provides sufficient information for identification of heterotic combinations.

## ACKNOWLEDGEMENTS

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# تجزیه و تحلیل GGE Biplot براساس دی آل جهت بهره‌برداری از شادابی هیبرید گیاه خشخاش (*Papawer semniferum* L.)

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## چکیده

از طرح پیوند دی آل (diallel cross) اغلب برای کسب اطلاعات در زمینهٔ آثار ژنتیکی، تخمـین تـوان ترکیبی عمومی و خاص( SCA و GCA) و شناسائی ترکیبات امیدبخش هیتروزیسی و همچنین زمینه‌هـای هیتروزیس استفاده مـی‌شـود. در خـلال تحقیـق کنـونی پیونـدهای هیتروزیسـی (Heterotic crosses) در راستای آلکالوئیدهای خاص( در گیاه خشخاس از نوع حاوی مـواد مخـدر) مـورد شناسـایی قـرار گرفتنـد. در خلال تحقیق از مدل Yan's GGE biplot و استفاده از داده‌های کامل دیالل ۵×۵ بهره‌برداری شـد. نتـایج اخذ شده با نتایج بدست آمده از تجزیه و تحلیـل از طریـق گریفینـگ (Griffing) (بـرای تأییـد دقـت مـدل GGE biplot یان (Yan)) مورد مقایسه قرار گرفت. والـدین Papline)A) و -B(NB5KR40-7/2 3) و (58/1) E به عنوان تلفیق شونده‌ها کلاً مطلوب شناخته شدند. کـراس‌هـای B×C و B×E و E×B از نظر مرفین، C×D و C×E از جهـت نـارکوتین و A×B و A×C و A×E بـه عنـوان ترکیبـات هیتروزیسـی مورد شناسائی واقع شدند. هیچ یک از کراس‌ها در ارتبـاط بـا کـودئین (Codeine) و تبـائین (Thebaine) هیتروزیس نبودند.