A triple test cross analysis to detect epistatic gene effects in cabbage (Brassica oleracea var. capitata L.): An updated methodology

Asma Jabeen *, Sanjay Chadha
CSK Himachal Pradesh Agricultural University, Palampur 176061, India

**ABSTRACT**

The cross-pollinated crop Brassica oleracea var. capitata L shows good heterotic heterosis at high output; better standing of the plants; early maturity; larger and more homogeneous heads; consistency of head compactness; and disease-tolerance in F1 hybrids. There is very limited information documented on the epistasis of essential cabbage characters. We expand the research in this study to include an upgraded test to cross-design for enrolling and estimating epistasis and other genetic variance components controlling head yield and component traits in cabbage. The data was obtained from 45 families produced by crossing 15 lines with three testers; SC 2008–09, E-1–3-1&2, and their single cross F1, was subjected to triple test cross analysis. The current study results confirmed “j + 1” form of epistasis which is a major component for all traits. The plant spread, non-wrapper leaves, nethead/grossweight, polar/equatorial diameter, marketable head yield per plot, iron content and dry matter lugged both “j + 1” and ‘i’ type with the predominance of the ‘i’ type of interaction. Except for head shape index, equatorial diameter, head compactness was more noticeable when observed in dominance component. The degree of dominance is in the partial range, but both the head shape index/compactness and equatorial diameter showed dominance. For maximum part, superiority was shown in both the directions. Appropriate breeding procedures are proposed to exploit the different forms of gene effects discovered for genetic improvement of head yield and quality traits.

**1. Introduction**

One of the most widely grown and widely eaten Brassica family vegetables is the cabbage (Brassica oleracea var. capitata L.) (Singh et al., 2021). Several brassicas (cabbage and others) are grown throughout the world. It is a good source of dietary fiber, minerals, including beta-carotene, vitamin C, and glucose, as well as folic acid and fructokinase (Liao et al., 2021). It is an essential component of culinary dishes throughout the world, eaten as a fresh salad and preserved by freezing, dehydration, or pickling (Posta and Berar, 2006). Besides being nutritionally rich, cabbage has medicinal uses as well like, anticarcinogenic properties, anti-inflammatory potential due to the presence of chemical compounds like glucosinolates, glutathione, isothiocyanates (Ghebramlak et al., 2006; Kopsell et al., 2004; Singh et al., 2009). Recognizing the importance and scope of cabbage as a commercial crop around the world, research efforts to improve and develop high yielding cabbage varieties/hybrids are needed. Crop enhancement programs’ effectiveness is highly reliant on the quality and scale of genetic components of variation. The selection of breeding procedure is primarily determined by knowledge of gene action regulating economic character. As a result, accurate estimates of such components are needed in order to construct an efficient breeding strategy. Many mating designs make the assumption that epistatic interactions are absent, which is often incorrect. The triple test-cross (TTC) research proposed by Kearsey and Jinks (1968) and their adaptation proposed by Jinks et al as are the most important design for the study of population genetic architecture (Jinks and Perkins, 1970). Based on TTC, there are few options for reliable tests and epistasis with unbiased estimation of both additive and dominant genetic components in the absence of epistasis with the widely used tests. Pooni and Jinks (1976) demonstrated that the triple test cross strategy outperformed the alternative
strategies for detecting epistasis. Furthermore, the method is unaffected by gene correlation, mating mechanism, or allelic frequency. This study was documented in limited studies and present study was to investigate the function of various genetic components within the head yield of inheritance and another horticulturally significant characters in cabbage.

2. Materials and methods

2.1. Precise location, materials and test design

This study was carried out within the premises of our department. The location of the research department was located at 32°6'E longitude and 76°3'E longitude and 1290.80 m at mean sea level. Agrochemically, the site is in Himachal Pradesh's mild hill region with the humid climate i.e., sub-temperature and a high rainfall of 2500 mm throughout the annum. Experimental materials used in this study 45-TTC progenies resulting from the mating of 15 inbred lines (females) with three testers (males), namely SC 2008–09 (P1), E-1-3-1&2 (P2), and their single cross F1. Table 1 shows the basic material used for producing triple test cross progenies, as well as their distinguishing characteristics. Jinks and Perkins (1970) suggested an updated TTC design, which was used for the crosses. SC 2008–09 and E-1-3-1&2 were crossed to each other and with 15 lines, resulting in plenty of F1 seeds, and 30 single crosses. Similarly, F1 as the male parent was crossed with 15 lines (females) to create 15 three-way crosses, yielding 45 triple test cross families (L1i, L2i, and L3i). During 2019–2020, this material was raised in triplets using Randomized Complete Block Design (October–March). Inter cultural operations were performed within the accordance with prescribed sets for vegetable crops (Sharma and Sirohi, 2003).

2.2. Observations on quantitative characteristics

The observations were made on ten competitive plants that were marked at random through the replications in each entry on distinct quantitative traits such as days to harvest, plant spread (cm), net weight of head (g), gross weight (g), non-wrapper leaves (n), stalk-length (cm), polar/equatorial diameter (cm), heading (%), head shape-index, compactness of head (g/cm$^3$), marketable heads per plot (n) and marketable head yield (kg/plot).

2.3. Examination in a laboratory

In fresh marketable heads, total soluble solids (°B), ascorbic acid (mg/100gs), iron content (mg/100gs), carotenoids (µg/100gs), vitamin B1 (µg/100gs), and dry matter (percent) were measured. TSS was determined using a hand refractometer. According to Rangnna (2007), ascorbic acid was measured using a colorimetric system with 2, 6-dichlorophenol indophenol dye. Rangnna (2007) developed a system for estimating iron content that used absorbance at 480 nm and iron requirements for the calibration curve. Carotenoids were collected in acetone solution, and spectrophotometer measurements were taken at 480, 663, and 645 nm OD. Sadasivam (1996) suggested a spectrophotometric approach for determining vitamin B1. The dry matter was calculated using the procedure described by Arora et al. (2008).

2.4. Statistical analysis

The statistical analysis for epistasis detection was performed with the existence of non-allelic interaction and epistasis can be calculated as the described method (Kearsey and Jinks, 1968). The testing and estimation of additive and dominance elements were performed with Jinks and Perkins (1970) and Singh and Chaudhary (1977) studies. The other stats were performed as per the documented study (Khan et al., 2019).

3. Results

In this current study, 15 lines and 3 testers of combined study variance for the parents and their specific 45 TTC hybrids were showed elevated substantial genotype variations for all traits (Table 2). The results obtained for 45 TTC hybrids were correlated with TTC analysis to estimate the numeric genetic components. The results which was showed in Table 3 was TTC analysis of variances confirmed the mean-square attributable to hybrids were highly important for all traits tested, indicating the existence of sufficient heterogeneity in the triple test cross progenies for detecting the breeding of recombination.

For all traits, the mean squares due to epistasis showed the existence of major non-allelic interactions (L1i + L2i – L3i) (Table 4). The existence of i$^i$ form of interaction (additive additive) for plant

### Table 1

| Lines/Testers | Internal colour of head | Shape of head in longitudinal section | Shelf life (in days) |
|---------------|------------------------|--------------------------------------|---------------------|
| I-105 (L1)   | Cream                  | Broad elliptic                       | 30                  |
| I-Suhasini (L2) | Yellowish             | Transverse elliptic                  | 30                  |
| I-Madhuri (L3) | Yellowish             | Transverse elliptic                  | 32                  |
| II-105 (L4)  | Yellowish              | Transverse narrow elliptic           | 33                  |
| II-Suhasini (L5) | Yellowish             | Transverse elliptic                  | 34                  |
| II-Madhuri (L6) | Cream                | Transverse elliptic                  | 30                  |
| III-105 (L7) | Yellowish             | Transverse narrow elliptic           | 33                  |
| III-Suhasini (L8) | Yellowish         | Transverse elliptic                  | 33                  |
| III-Madhuri (L9) | Yellowish             | Transverse elliptic                  | 28                  |
| Glory-I-Suhasini (L10) | Yellowish         | Transverse elliptic                  | 30                  |
| Glory-I-Madhuri (L11) | Yellowish         | Transverse elliptic                  | 33                  |
| Glory-7-Suhasini (L12) | Cream            | Broad elliptic                       | 33                  |
| Glory-7-Madhuri (L13) | Cream             | Transverse elliptic                  | 30                  |
| GA(P)-105 (L14) | Cream               | Transverse elliptic                  | 30                  |
| GA(P)-Suhasini (L15) | Yellowish         | Broad elliptic                       | 30                  |
| GA(P)-Madhuri (L16) | Yellowish         | Transverse elliptic                  | 33                  |
| SC 2008–09 (P1) | Yellowish           | Transverse elliptic                  | 30                  |
| E-1-3-1&2 (P2) | Whitish              | Transverse elliptic                  | 32                  |
| F1 (P1 × P2)  | Yellowish             | Transverse elliptic                  | 28                  |

### Table 2

Analysis of variance for the Randomized Complete Block Design for head yield and related traits in cabbage.

| Source of variation | Mean squares due to |
|---------------------|---------------------|
| Traits df           | Replications 2 | Treatments 62 | Error 124 |
| Days to harvest     | 7.871             | 162.782*       | 2.708     |
| Plant spread (cm)   | 2.116             | 25.212*        | 2.423     |
| Stalk length (cm)   | 0.095             | 0.807*         | 0.102     |
| Number of non-wrapper leaves | 13.334 | 3.127* | 1.018 |
| Gross weight (g)    | 635.069           | 80626.990*     | 65.085    |
| Net head weight (g) | 57.815            | 47486.950*     | 43.761    |
| Polar diameter (cm) | 0.135             | 2.636*         | 0.059     |
| Equatorial diameter (cm) | 0.102 | 2.001* | 0.054    |
| Head shape index    | 0.003             | 0.012*         | 0.002     |
| Head compactness (g/cm$^3$) | 0.637 | 89.781* | 0.725    |
| Marketable heads per plot | 0.958 | 1.607* | 0.592    |
| Heading (%)         | 66.498            | 111.601*       | 41.100    |
| Marketable head yield per plot (kg) | 0.252 | 5.884* | 0.249    |
| Ascorbic acid content (mg/100g) | 0.000 | 0.012* | 0.000    |
| Carotenoid content (µg/100g) | 30.820 | 18946.970* | 53.916 |
| Total soluble solids (°Brix) | 2.271 | 1.735* | 0.148    |
| Iron content (mg/100g) | 0.876 | 26.476* | 1.889    |
| Dry matter (%)      | 0.020             | 0.622*         | 0.010     |
| Vitamin B1 content (µg/100g) | 4.658 | 109.639* | 1.889    |
spread, gross weight, non-wraper leaves, net head-weight, polar-diameter, equatorial-diameter, marketable head yield per plot, head shape-index, iron content, and dry matter was discovered by more mean square partitioning due to epistasis into ‘i’ and ‘j + l’. On the other hand, all of the characters examined had ‘j + l’ form of relationship (additive dominance and dominance dominance). As a result, it is obvious that the role of epistasis should not be overlooked when developing a breeding program to enhance commercially relevant traits. Epistasis is an essential for genetic variance: it’s zygosity cannot be accurately calculated if it is neglected. If that is the case, multiplicative and dominant parameters will be underestimated, as well as additive and dominant/dominant conclusions will be erroneously estimated.

4. Discussion

Earlier researchers, such as Sharma et al., Atter et al., Meena et al., and Singh et al, have documented considerable variability in their respective cabbage materials for the various collection of characters studied (Sharma, 2001; Atter et al., 2009; Meena et al., 2009; Singh et al., 2013). Estimates of mean squares due to quantities (measuring D component) and disparities (measuring H component) revealed that additive and dominance genetic variances were important for all characters except marketable heads per plot and heading percentage, for which the D component was non-significant. This demonstrated the significance of both components in regulating these characteristics. Because of the greater magnitude of additive gene action, fixable gene action was observed for the majority of the traits. However, due to the higher magnitude of the dominance variable, the non-fixable form of gene action was predominant for equatorial diameter, head shape index, and head compactness. The average degree of dominance (H/D) 1/2 for the majority of the traits was in the range of partial dominance. Overdominance was observed for equatorial diameter, head shape index, and head compactness, indicating the significance of both additive and non-additive gene effects. For most of the characters examined, the r estimates indicated ambi-directional dominance, with declining alleles more frequently dominant than increasing alleles (Table 5). Soliman (2002), Thakur, and Vidyasagar (Reshma et al., 2018) have all expressed similar viewpoints.

Where gene activity is involved in the expression of traits in both additive and non-additive forms, the proposal was made to use reciprocal recurrent selection to improve the characteristics. The main additive action in head yield and most of its constituent characteristics was successfully established in this paper, which is contrary to previous reports that non-additive gene action was dominant in head yield and in most of its traits through diallel line testing designs (Parkash et al., 2003; Pathak and Kumar, 2007; Singh et al., 2011, 2018; Kibar et al., 2015). As a result, this demonstrates the dependability of the triple test cross mating design in accurately evaluating gene action of biometrical traits.

Table 3

| Source of variation | Mean squares due to Replications | Hybrids | Error |
|---------------------|----------------------------------|---------|-------|
| Traits df          |                                  |         |       |
| Days to harvest    | 1.844                            | 152.196e | 2.996 |
| Plant spread (cm)  | 1.057                            | 26.892e  | 2.669 |
| Stalk length (cm)  | 0.075                            | 0.667e   | 0.104 |
| Number of non-wraper leaves | 19.788    | 3.865e  | 1.121 |
| Gross weight (g)   | 360.252                           | 61526.00e | 64.615 |
| Net head weight (g) | 24.067                     | 23836.06e | 52.188 |
| Polar diameter (cm) | 0.043                     | 2.600e  | 0.032 |
| Equatorial diameter (cm) | 0.031                | 1.434e   | 0.028 |
| Head shape index   | 0.001                            | 0.015e   | 0.001 |
| Head compactness (g/cm³) | 0.090                   | 60.382e  | 0.797 |
| Marketable heads per plot | 1.096                  | 1.416e  | 0.611 |
| Heading (%)        | 76.098                           | 98.344e  | 42.443 |
| Marketable head yield per plot (kg) | 0.343                  | 2.864e  | 0.299 |
| Ascorbic acid content (mg/100 g) | 1.501                   | 29.250e  | 1.054 |
| Total soluble solids (Brx) | 3.110                 | 1.699e  | 0.155 |
| Carotenoid content (ug/100 g) | 79.954                  | 22136.570e | 54.114 |
| Iron content (mg/100 g) | 0.000                     | 0.050e  | 0.000 |
| Dry matter (%)     | 0.015                            | 0.635e   | 0.011 |
| Vitamin B1 content (ug/100 g) | 4.946                  | 113.988e | 1.942 |

1 Significant at P ≤ 0.05.

Table 4

| Source of variation | Epistasis | i-type interaction | (j + l) type interaction | Epistasis × replication | i-type × replication | (j + l) type × replication |
|---------------------|-----------|--------------------|--------------------------|-------------------------|----------------------|---------------------------|
| Traits df          | 15        | 1                  | 14                       | 30                      | 2                    | 28                        |
| Days to harvest    | 754.405e  | 449.500            | 776.542e                 | 24.294                  | 44.564               | 22.846                     |
| Plant spread (cm)  | 164.854e  | 426.703e           | 146.150e                 | 18.618                  | 14.311               | 18.925                     |
| Stalk length (cm)  | 3.581e    | 2.218              | 3.678e                   | 0.434                   | 2.007                | 0.321                      |
| Number of non-wraper leaves | 27.687e    | 101.971e           | 22.375                   | 8.076                   | 4.205                | 8.353                      |
| Gross weight (g)   | 483014.333e | 2586622.250e       | 354183.196e              | 337.978                 | 965.437              | 293.159                    |
| Net head weight (g) | 195279.044e | 1883752.125e       | 74673.824e               | 531.867                 | 2330.108             | 403.420                    |
| Polar diameter (cm) | 23.289e   | 149.459            | 14.276                   | 0.256                   | 0.272                | 0.255                      |
| Equatorial diameter (cm) | 5.661e       | 11.430e            | 5.258e                   | 0.276                   | 0.323                | 0.273                      |
| Head shape index   | 0.122     | 0.527e             | 0.093                    | 0.006                   | 0.006                | 0.006                      |
| Head compactness (g/cm³) | 373.135e   | 60.152             | 395.494e                 | 2.800                   | 0.822                | 2.941                      |
| Marketable heads per plot | 8.133e    | 10.755             | 7.446e                   | 194.402                 | 56.949               | 204.211                    |
| Marketable head yield per plot (kg) | 18.175e    | 130.629e           | 10.143e                  | 60.177                 | 5.897                | 8.404                      |

1 Significant at P ≤ 0.05.
A. Jabeen and S. Chadha

Saudi Journal of Biological Sciences 28 (2021) 6153–6157

5. Conclusion

The present study was concluded as the analysis of variance shows that the material under study produced a significant amount of genetic variability. The presence of non-allelic interactions stressed that it is difficult to imagine a case in which epistasis might be considered missing and thus should not be overlooked as this would lead to either over or under estimation of additive and dominance components of variation. In the early generations, breeding procedures such as selection could be useful for improving those characters that showed a preponderance of additive gene effects. The current result also demonstrated the significance of additive dominance (j) and dominance (l) epistasis in the inheritance of head yield and related traits. Since they are non-fixable, the dominance part, as well as the ‘i’ and ‘j’ types of epistasis, can be exploited by developing high yielding hybrids through heterosis breeding.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Arora, D., Jindal, S.K., Ghai, T., 2008. Gene action for some biochemical traits in okra (Abelmoschus esculentus (L.) Moench). Veg Sci 35 (2), 180–184.
Atter, R., Sharma, K., Sundourri, A., 2009. Genetic variability, heritability and genetic advance studies in cabbage (Brassica oleracea var. capitata L.). Indian J. Plant Genetic Resour. 22 (1), 62–65.
Ghebramlak, A., Sharma, S., Rattan, R., 2006. Genetic variability and correlation studies for quality characters in cabbage (Brassica oleracea L. var. capitata). Genet Resour. 22 (1), 62–65
Jinks, J., Perkins, J., 1970. A general method for the detection of additive, dominance and epistatic components of variation III. F2 and backcross populations. Heredity 25, 419–429.
Kearse, M., Jinks, J., 1968. A general method of detecting additive, dominance and epistatic variation for metrical traits I. Theory. Heredity 23 (3), 403–409.
Khan, L.A., Jahan, P., Hasan, Q., Rao, P., 2019. Genetic confirmation of T2DM meta-analysis variants studied in gestational diabetes mellitus in an Indian population. Diab. Metab. Syndr. 13 (1), 688–694. https://doi.org/10.1016/j.dsx.2018.11.035.
Kilar, B., Karaasag, O., Kar, H., 2015. Heterosis de las características productivas de la cabeza del repollo (Brassica oleracea var. capitata). Ciencia e investigación agraria 42 (2), 205–216.
Koppell, D.A., Koppell, D.E., Lefsrud, M.G., Curran-Celentano, J., Dukach, L.E., 2004. Variation in lutein, β-carotene, and chlorophyll concentrations among Brassica oleracea cultivars and seasons. HortScience 39 (2), 361–364.
Liao, X., Rao, S., Yu, T., Zhu, Z., Yang, X., Xue, H., Gou, Y., Cheng, S., Xu, F., 2021. Selection trait improved the Se accumulation, nutrient quality and antioxidant system of cabbage (Brassica oleracea var. capitata L.). Plant Signal Behav.. 1907042.
Meeha, M., Ram, R., Lata, R., 2009. Genetic variability and correlation studies for some quantitative traits in cabbage (Brassica oleracea var. capitata L.). Indian J. Agric. Sci. 77 (7), 97–100.
Parkash, C., Verma, T., Kumar, P., 2003. Genetic analysis of cabbage (Brassica oleracea var capitata) using self-incompatible lines. Indian J. Agric. Sci. 73 (7), 412–413.
Pathak, S., Kumar, J., Sagar, V., Verma, S., 2007. Heterosis and combining ability for marketable yield and component traits in cabbage (Brassica oleracea var capitata). Indian J. Agric. Sci. 77 (2), 97–100.
Poorn, H., Jinks, J., 1976. The efficiency and optimal size of triple test cross designs for detecting epistatic variation. Heredity 36 (2), 215–227.
Posta, G., Berar, V., 2006. Researches concerning the yield performances of the early cabbage hybrids cultivated in the field conditions. Bulentinul Universitatii de Stiinte Agroicole si Medicina Veterinara, Cluj-Napoca Seria Horticultura 63, 101–105.
Rangina, S., 2007. Handbook of Analysis and Quality Control for Fruits and Vegetable Products. Tata Mcgrawhill.
Reshma, V., Adarsh, M., Dhanush, K., 2018. Development of hybrids in cole crops: a review. Plant Arch. 18 (1), 1–11.
Sadasivam, S., 1996. Biochemical Methods. New Age International.
Sharma, S., 2003. Cabbage. In: Sirohi, P.S. (Ed.), Vegetable Crop Production. Division of Vegetable Science. IARI, New Delhi.
Sharma, D., 2001. Horticultural Evaluation and Variability Studies in Some Cabbage Genotypes. College of Horticulture Dr. Yashwant Singh Parmar University of Horticulture.
Singh, R.K., Chaudhary, B.D., 1977. Biometrical methods in quantitative genetic analysis. Biometrical methods in quantitative genetic analysis.
Singh, S., Bhatia, R., Kumar, R., Sharma, K., Dash, S., Dey, S.S., 2018. Cytoplastic male sterile doubled haploid lines with desirable combining ability enhances the concentration of important antioxidant attributes in Brassica oleracea. Euphytica 214 (11), 1–19.
Singh, B., Sharma, S., Singh, B., 2009. Heterosis for mineral elements in single cross-hybrids of cabbage (Brassica oleracea var capitata L.). Scientia Horticulturae 122 (1), 32–36.
Singh, B., Sharma, S., Singh, B., 2011. Combining ability for antioxidants and economic traits in cabbage. Indian J. Hortic. 68 (4), 495–497.
Singh, B., Sharma, S., Singh, B., 2013. Genetic variability, inheritance and correlation for minor contents in cabbage (Brassica oleracea var. capitata L.). J. Horticult. Res. 21 (1).
Singh, M.K., Singh, A.K., Singh, R.K., Mishra, D., Singh, B., Pal, A., Singh, B., 2021. Effect of foliar application of various micronutrients on yield and economics of cabbage (Brassica oleracea var. capitata L.). J. Pharmac Phytochem. 10 (1), 1265–1267.

Solieman, T., 2002. Genetical studies on some quantitative characters of developed inbred lines of Balady cultivar of cabbage, Brassica oleracea variety capitata L. and their hybrid combinations. I.-general performances of the genetic populations, detected heterosis and potence ratio estimates. Alexandria J. Agric. Res. (Egypt).