Estimation of heterosis in drought tolerant hybrids of cocoa

JUBY BABY*[1], MIMIMOL J.S.[1], B. SUMA[1], A.V. SANTHOSH KUMAR[2]
[1]Cocoa Research Centre, KAU, Thrissur
[2] Department of Forest Biology and Tree Improvement
Kerala Agricultural University
College of Horticulture, Vellanikkara, Thrissur
INDIA
*Corresponding author; jubybaby93@gmail.com

Abstract: - Production of hybrids may be the best way to exploit the heterosis in cocoa and wide crossing to exploit hybrid vigour is the most accepted method of cocoa breeding. Hybrid seedlings are generally preferred as planting material in view of right plant architecture, ease in management, less cost involved and resistance to abiotic and biotic stress.

Key-words:- *Theobroma cacao*, Relative heterosis, Heterobeltiosis, Nitrate reductase, Chlorophyll, Photosynthesis

1 Introduction

Cocoa (*Theobroma cacao* L.) is a perennial crop plant belonging to the family Malvaceae and which is a native of Amazon river basin. It is mainly grown in humid tropical regions which is ideal for it's development.

Cocoa has been consumed as a beverage crop even before the introduction of tea and coffee and has been a part of many ancient cultures [1]. The literal meaning of cocoa is the "Food of Gods" as the plant was worshipped in many cultures and the people thought this plant as a gift from the heavens. Cocoa originated in the river basin of Amazon and it is obvious from its centre of origin that it requires an ample amount of water for its cultivation and cannot withstand long periods of drought.

The development of cocoa can be divided into two phases, before and after development of hybrids [2]. Cocoa, has been known to exhibit strong heterosis for yield and yield contributing characters

[3]. Even though there are many studies being carried out in cocoa, the research lies very behind in exploiting the full potential of cocoa. Many physiological and genetic investigation have exposed that the full yield potential of cocoa has not been fully exploited [4]. The hybrids when analysed are found to be early bearing and can also tolerate biotic and abiotic stress than clones [5]. Apart from pests and diseases, water stress is one of the major factors that needs to be taken care of. The present study was carried out at Cocoa Research Centre, Kerala Agricultural University in order to estimate the performance of hybrid seedlings bred for drought tolerance over their parents.

2 Materials and methods

Four genotypes (M 13.12, G I 5.9, G II 19.5 and G VI 55) identified to be tolerant to drought in preliminary studies conducted at Cocoa Research Centre (CRC), by screening existing germplasm [6] were used as parents for hybridisation.

These genotypes were crossed in all possible combination by a method described by Mallika *et al*. [7]. A total of 120 hybrids exhibiting initial vigour from various crosses were selected for further analysis. Seedlings at their fifth moth stage were then subjected to drought stress by gravimetric method for a period of two weeks. They were kept at 40 per cent field capacity and then after two weeks, were subjected to various biochemical and physiological studies.

To evaluate the superiority of hybrids over parents, relative heterosis and heterobeltiosis was estimated using the formula:

1. Relative heterosis = $\frac{F_1 - \overline{MP}}{\overline{MP}}$ --------(1)

2. Heterobeltiosis = $\frac{F_1 - \overline{BP}}{\overline{BP}}$ --------(2)

Where,

MP- value of mid- parental value
BP- value of better parent

The significant value was estimated using the t-value:

3. Relative heterosis (di) = $\frac{\sqrt{3\ EMS}}{\sqrt{2r}}$ -------(3)

t- value for di = $\frac{F_1 - MP}{SE}$ -------(4)

4. Heterobeltiosis (dii) = $\frac{\sqrt{2\ EMS}}{\sqrt{r}}$ --------(5)

t- value for dii = $\frac{F_1 - BP}{SE}$ --------(6)

SE- Standard error

3 Results and discussion

Relative heterosis and heterobeltiosis of the 11 sets of crosses were calculated as per cent increase or decrease over mid parent and better parent respectively. The results are represented in Table 1 and 2.

3.1 Heterosis among hybrids for biochemical parameters

3.1.1 Proline

Proline acts as a compatible osmolyte since it accumulates in high concentrations without damaging cellular macromolecules. Proline can also serve as a nitrogen carbon source in the cell [8].

High mid parent heterosis was shown by hybrid H43 (1246.29%) to as low as 2.67 per cent in hybrid H21. However, the majority of hybrids showed superiority over the mid parental value having high significant positive effects. Negative significant relative heterosis was shown by some hybrids, the highest being in hybrid H44 (-59.13%) (Table 1).

High effect of heterosis over the better parent was also shown by hybrids, being as high as 1220% in H43 to as low as 0.61 per cent in H41. High negative heterobeltiosis having significant effect was shown by hybrid H44 (-59.92%) (Table 2).

High amount of proline is observed in hybrids and comparatively low amount was seen in parents as well as control. In soyabean, a similar work was done and the hybrids showed high proline content which expressed dominant and heterotic mode of inheritance, which was recommended as potential drought resistant lines [9].

3.1.2 Nitrate reductase activity

NRase is intimately associated with plant growth and development [10].

In case of NRA, hybrid H115 showed maximum relative heterosis (221.31%) to as low as 1.96 per cent in hybrid H13. High negative significant heterotic effect was shown by hybrid H17 (-91.70%) (Table 1).

In case of heterobeltiosis, H115 only showed high heterotic effect over the better parent (508.14 times) and the lowest effect was shown by hybrid H111 (4.54 %). Significant negative effect was shown by hybrid H116 (-787.67 %) (Table 2). In the case of cocoa hybrids, high heterotic effects was shown by many hybrids which was even more than 100 per cent heterosis indicating its superiority over the parents.

3.1.3 Superoxide dismutase (SOD)

Superoxide Dismutase are particularly important in defence mechanisms and cross-tolerance phenomena, enabling a general acclimation of plants to stressful conditions. H_2O_2, a breakdown product of SOD, has long been recognized as a signal-transducing molecule in the activation of defence responses in plants under stress [11]. Hence, SOD serves as the first line defence for plants under stress conditions.

Here, the hybrid H10 showed maximum mid- parent heterosis of about 95.38 per cent and the lowest effect was shown by hybrid 112 (1.38%). The negative significant effect was shown by hybrid H108 (-49.71%) (Table 1).

Hybrid H10 showed highest heterotic effect over the better parent having about 75.58 per cent superiority and hybrid H58 showed the lowest value of about 0.46 per cent indicating it’s inferiority over the better parent. High negative significant heterobeltiotic effect was shown by H108 (-51.55 %) (Table 2).

In the case of rice, the increase in SOD activity was higher in female parent under water stress when compared to the male parent and hybrid [12].

3.1.4 Glycine betaine

Glycine betaine effectively stabilizes the quaternary structure of enzymes and complex proteins, and it maintains the highly ordered state of membranes at non- physiological temperature and concentrations [13]. In majority of hybrids, the amount of glycine betaine was high.

High mid parent heterosis was shown by hybrid H103 (72.37%) to as low as 0.25 per cent in hybrid H105. Negative significant relative was shown by some hybrids, the highest being in hybrid H3 (-48.48%) (Table 1).

High effect of hybrids over the better parent was also shown by hybrids, being as high as 69.20 times more superior in H103 to as low as

1.89 per cent in H94. High negative heterobeltiosis having significant effect was shown by hybrid H3 (-50.88%) (Table 2).

3.2 Heterosis among hybrids for physiological parameters

3.2.1 Chlorophyll stability Index

Chlorophyll stability Index is one of the factors that contribute to assessing the drought tolerant conditions in plants.

Hybrid H26 showed maximum mid- parent heterosis of about 65.83 per cent and the lowest effect was shown by hybrid H100 (0.6%). The negative significant effect was shown by hybrid H72 (-36.10 %) (Table 1).

Hybrid H26 showed highest heterotic effect over the better parent having about 64.92 per cent superiority and hybrid H65 showed the lowest value of about 0.3 per cent indicating it's inferiority over the better parent. High negative significant heterobeltiotic effect was shown by H72 (-37.60 %) (Table 2). In cashew, chlorophyll stability index of drought tolerant accessions was higher than sensitive varieties [14].

3.2.2 Cell membrane stability (CMS)

Biological membranes are the first target of many abiotic stresses and it is generally accepted that the maintenance of integrity and stability of membranes under water stress is a major component of drought tolerance with respect to plants [15].

In case of CMS, hybrid H37 showed maximum relative heterosis (45.38%) to as low as 0.06 per cent in hybrid H41. High negative significant heterotic effect was shown by hybrid H58 (-47.95%) (Table 1).

In case of heterobeltiosis, H37 showed high heterotic effect over the better parent (40.53%) and the lowest effect was shown by hybrid H115 (1.56 %). Significant negative effect was shown by hybrid H58 (-51.13%) (Table 2). The membrane stability of leaves is considered as the most important trait to screen the germplasm for drought tolerance [16].

3.2.3 Relative water content

When analysed, the hybrids recorded maximum heterotic effects (47%) and were being more tolerant to others for relative water content. Relative magnitude of heterosis for relative water content was higher in stress than in non-stress conditions [17]. 51.87 per cent mid parent heterosis for relative water content has been observed in some cases [18].

In the hybrids, Hybrid H85 showed maximum mid- parent heterosis of about 72.16 per cent and the lowest effect was shown by hybrid H118 (0.28%). The negative significant effect was shown by hybrid H116 (-55.93 %) (Table 1).

Hybrid H13 showed highest heterotic effect over the better parent having about 58.00 per cent superiority and hybrid H89 showed the lowest value of about 5.75 per cent indicating it's inferiority over the better parent. High negative significant heterobeltiotic effect was shown by H116 (-56.11 %) (Table 2). It indicated that the hybrids had more water retention capacity than the parental and the control genotypes.

3.2.4 Photosynthesis

The ability of crop plants to acclimate to different environments is directly or indirectly associated with the plant's ability to acclimate at the level of photosynthesis, which in turn affects biochemical and physiological processes and, consequently, the growth and yield of the whole plant [19].

In the hybrids, high mid parent heterosis was shown by hybrid H20 (193.28 %) to as low as 1.06 per cent in hybrid H61. Negative significant relative was shown by some hybrids, the highest being in hybrid H14 (-17.66%) (Table 1).

High heterotic effect over the better parent was also shown by hybrids, being as high as 158.54 per cent in H20 to as low as 0.41 per cent in H88. High negative heterobeltiosis having significant effect was shown by hybrid H44 (-29.20%) (Table 2). This indicated the superiority of hybrids in photosynthetic rate even during drought conditions.

3.2.5. Transpiration rate

Water stress has been known to reduce the transpiration rate in plants. In case of transpiration rate, hybrid H23 showed maximum relative heterosis (407.73%) to as low as 2.86 per cent in hybrid H109. High negative significant heterotic effect was shown by hybrid H42 (-52.55%) (Table 1).

When heterobeltiosis was estimated, H23 only showed high heterotic effect over the better parent (466.67 %) and the lowest effect was shown by hybrid H70 (1.11 %). Significant negative effect was shown by hybrid H118 (-44.36 %) (Table 2).

Three accessions of cocoa (NC 23, NC 29 and NC 39) showed 54 to 59 percent decrease in transpiration rate under stress conditions as compared to plants under irrigation [20].

3.2.6 Chlorophyll content

Hybrid H97 showed maximum mid- parent heterosis of about 80.95 per cent and the lowest effect was shown by hybrid H21 (0.57%). The negative significant effect was shown by hybrid H45 (-34.89 %) (Table 1).

Hybrid H97 showed highest heterotic effect over the better parent having about 53.87 per cent superiority and hybrid H3 showed the lowest value of about 0.69 per cent indicating it's inferiority over the better parent. High negative significant heterobeltiotic effect was shown by H80 (-39.43 %) (Table 2).

Chlorophyll content was estimated for parents as well as F1 hybrids in Chinese cabbage and it was observed that the amount of chlorophyll in F1 hybrids was more than in the parental genotypes because of the increased size and number of cells resulting in an increased leaf area and fresh weight in the F1 hybrid. The amount of chlorophyll was up-regulated in F1 hybrids than the mid parental value indicating the superiority of hybrids among the parents [21].

3.2.7 Leaf temperature

Leaf temperature is an important factor in controlling leaf water status under water deficit conditions [22]. Generally under drought stress, the leaf temperature increases. However, in the experiment, the hybrids were not having any significant rise in leaf temperature and were not having any significant effect either on the mid parental value or the better parent value. Hence, the values were insignificant.

4 Conclusion

From Table 1 and 2, it was concluded that majority of hybrids expressed heterotic values for four characters *viz.* photosynthetic rate, chlorophyll stability index, proline and chlorophyll content in case of mid parent heterosis and more hybrids expressed superiority over the better parent value for the charecters photosynthesis, chlorophyll stability index, proline and superoxide dismutase. The biochemical and physiological parameters when considered, majority of hybrids expressed superiority over parents indicating that heterosis breeding can be exploited in cocoa for drought tolerance breeding. The segregating behaviour in F1 generation producing hybrids of two extreme is due to heterozygotic nature of parents

From table 3, it was observed that crosses M 13.12 x G I 5.9, G II 19.5 x G I 5.9, G II 19.5 x M 13.12 and G II 19.5 x G VI 55 were showing more relative as well as heterobeltiotic effect. These hybrids has to be further evaluated under field condition to know their actual performance. The hybrids identified in table 3 and 4 can be used in breeding programmes to enhance the drought tolerant trait by making these hybrids as parents which will give rise to more heterotic progenies having superiority over their parents.

References:

[1] Roberto Verna, The history and science of chocolate. The *Malaysian Journal of Pathology,* Vol.35, No.2, 2013, pp.111–121.

[2] Dias, L. A. S., Marita, J., Cruz, C. D., Barros, E.G. and Salomao, T. M. F., Genetic distances and it association with heterosis in cocoa. *Brazilian Archives of Biology and Technology,* Vol.46, 2003, pp.339-347.

[3] Atanda, O. A. and Toxopeus, H., Approved case of heterosis, Proceedings of the Third International Cocoa Research Conference, *Accra*, 1971, pp.545–551.

[4] Bertus, E., How to select superior mother trees in heterogenous progenies for more productive clone or hybrid varieties. *Ingenic Newsletter,* Vol.9, 2004, pp.2-3.

[5] Edwin, J. and Masters, W., Genetic improvement and cocoa yields in Ghana. *Experimental Agriculture*, Vol.41, 2005, pp.491–503.

[6] Binimol, B., Identification of drought tolerant cocoa types. M.Sc. (Ag.) Thesis, Kerala Agricultural University, Vellanikkara, Thrissur, 2005, pp.95.

[7] Mallika, V. K., Prasannakumari Amma, S. and Vikraman Nair R., Crop improvement in cocoa (*Theobroma cacao* L.). Extended summaries of papers presented in the National seminar on technologies for enhancing productivity in cocoa, 29-30 November, 2002. Bhat, C. R., Balasimha, D. and Jayasankar, S. (Eds.), Central

Plantation Crops Research Institute, Regional station, Vittal, Karnataka, 2002, pp.19-27.

[8] Kishor, P. B. K., Sangama, S., Amrutha, R. N., Laxmi, P. S., Naidu, K. R. and Rao, K. S., Regulation of proline biosynthesis degradation, uptake and transport in higher plants: its implications in plant growth and abiotic stress tolerance. *Current Science*, Vol.88, No.3, 2005, pp.424-438.

[9] Sanja T. K., Đorđe R. M., Milan T. P., Marija M. K., Jegor A. M., and Aleksandar, D. I., Nitrogen metabolism enzymes, soluble protein and free proline content in soybean genotypes and their F1 hybrids. *Proc Nat Sci, MaticaSrpska Novi Sad*, Vol.115, 2008, pp.21-26.

[10] Sinha, S. K. and J. D. Nicholas., Nitrate Reductase in Relation to Drought. In: Physiology and Biochemistry of Drought Resistance, Palag, L. G. and L. D. Aspinal (Eds.). Academic Press, Sydney, Australia. 1981.

[11] Foyer, C. H. and Noctor, G., Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. *Physiologia Plantarum*, Vol.119, 2003, pp.355–364.

[12] D. Nageswara Rao, S. R. Voleti, D. Subrahmanyam, P. Raghuveer Rao and M. S. Ramesha, Heterosis for antioxidant enzymes in rice hybrid under abiotic stress conditions, *Indian Journal of Plant Physiology*, Vol.17, No.1, 2012, pp.154-157.

[13] Papageorgiou, G. C. and Murata, N., The unusually strong stabilizing effects of glycine betaine on the structure and function of the oxygen-evolving Photosystem-II complex, *Photosynthesis Research*, Vol.44, 1995, pp. 243–252.

[14] Latha, A. 1998. Varietal reaction to nutrient and moisture stress in cashew (*Anacardium occidentale* L.). Ph.D. Thesis, Kerala Agricultural University, Vellanikkara, Thrissur, 1998, pp.177.

[15] Bajji, M., Kinet, J. M., Luts, S., The use of electrolytic leakage method for assessing the cell membrane stability as a water stress tolerance test in durum wheat, *Plant Growth Regulation*, Vol.36, 2002, pp.61–70.

[16] Dhanda S. S., G. S. Sethi and R. K. Behl, Indices of drought tolerance in wheat genotypes at early stages of plant growth, *Journal of Agronomy and Crop Science*, Vol.190, 2004, pp.6–12.

[17] W. A. Jatoi, M. J. Baloch, N. U. Khan, M. Munir, A. A. Khakwani, N. F. Vessar, S. A. Panhwar and S. Gul, Heterosis for yield and physiological traits in wheat under water stress conditions, *Journal of Animal and Plant Sciences*, Vol.24, No.1, 2014, pp.252-261.

[18] Ganapathy, S. and S. K. Ganesh, Heterosis analysis for physio-morphological traits in relation to drought tolerance in rice (*O. sativa* L.), *World Journal of Agricultural Sciences*, Vol.4, 2008, pp.623-629.

[19] Chandra, S., Energy exchange characteristics as an indicator of biomass production potential in tree species, *Belgian Journal of Botany*, Vol.136, No.1, 2003, pp.45–51.

[20] Balasimha, D., Subramonian, N., and Subbaiah, C.C., Leaf characteristics in cocoa accessions, *Café Cacao*, Vol.29, 1985, pp.95-98.

[21] Natsumi, S., Takahiro, K., Hua Ying, Motoki, S., Mikiko, K., Hiroshi, A., Keiichi, O., Makoto, K., Jennifer M. T., Hitoshi, S., W. J. Peacock., Elizabeth S. D. and Ryo F., Molecular and cellular characteristics of hybrid vigour in a commercial hybrid of Chinese cabbage, BMC *Plant Biology*, 2016, pp.16-45.

[22] Leopold A. C., W. Q. Sun and L. Bernal-Lugo, The glassy state in seeds: analysis and function, *Seed Science Research*, Vol.4, 1994, pp.267-274.