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GENOTYPIC STABILITY IN DOUBLE HAPLOID LINES (DH) OF ORNAMENTAL KALE (*BRASSICA OLERACEA* VAR. *ACEPHALA* DC.) FOR KEY FLORICULTURAL TRAITS

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ABSTRACT

Kale (*Brassica oleracea* var. *acephala*), a member of the genus Brassica within the family Cruciferae, is widely valued for its versatility and ornamental potential. Ornamental kale, in particular, exhibits vibrant, colorful foliage under cold temperatures, making it a desirable choice for landscape and garden applications from late autumn to early spring. Its aesthetic appeal and extended foliage longevity further enhance its utility. Due to the strong expression of heterosis, most commercial ornamental kale cultivars are F_a hybrids. This study aimed to evaluate the phenotypic stability of 13 doubled haploid (DH) lines, previously developed through microspore culture, along with two commercial check varieties over three years. The goal was to identify genotypes exhibiting uniform performance across diverse environmental conditions for key floricultural traits. Stability analysis was conducted to assess the adaptability and performance consistency of these genotypes in varying environmental contexts. The analysis of variance revealed significant differences among genotypes and across environments for all traits examined, underscoring the presence of substantial genetic diversity. Notably, the DH lines KtDH-57 and KtDH-19 displayed stable and predictable performance across most evaluated traits. These genotypes emerged as promising candidates for commercial cultivation, particularly in the mid-hill temperate regions of Himachal Pradesh. This study further highlights the value of utilizing doubled haploid lines in ornamental kale breeding programs. Their inherent homozygosity and homogeneity, far from being limiting factors, proved advantageous in developing stable and high-performing genotypes. These findings reinforce the potential of DH technology as a robust tool for enhancing breeding efficiency in ornamental kale.

Key words : Ornamental kale, Stability, Doubled haploid, Environments, Floricultural traits.

Introduction

Ornamental kale (*Brassica oleracea* var. *acephala* DC.), a cultivated variety of *Brassica oleracea* (CC, 2n = 18), is a valuable ornamental foliage plant distinguished by its diverse leaf colours and forms. It is widely used in landscaping, as a potted plant and for cut foliage due to its aesthetic appeal. Notably, ornamental kale exhibits significant tolerance to frost and chilling temperatures, allowing vigorous growth in cold climates (Li and Yu, 2006). These traits make it an ideal bedding plant for colder seasons and regions. However, compared to other Brassica crops, the commercial availability of ornamental kale varieties is limited, and a scarcity of breeding resources poses a significant challenge to germplasm innovation and the development of new cultivars. Heterosis, or hybrid vigor, is well-documented in *B. oleracea*, making hybridization a common breeding strategy for producing F_1 hybrids. This approach not only ensures uniformity and superior performance but also facilitates intellectual property protection of new varieties. The development of homozygous inbred lines, critical for producing consistent hybrid plants and enabling genetic studies (Dunwell, 2010), traditionally requires six to eight generations of selfing or sib-mating. This process is labour-intensive and time-consuming.

The use of doubled haploid (DH) technology has significantly accelerated inbred line development, reducing the time required to achieve homozygosity to just one or two generations (Prigge *et al*., 2011). In comparison, classical methods such as pedigree breeding achieve 96.9% homozygosity only after six to ten generations of selfing heterozygous material (Allard, 1960). Moreover, lines developed through the DH method exhibit agronomic performance comparable to those produced using methods like pedigree, single seed descent (Park *et al*., 1976), or bulk breeding. However, while DH-derived lines are completely homozygous and homogeneous, heterozygosity has been reported to confer advantages under suboptimal conditions, suggesting potential limitations of complete homozygosity (Allard and Bradshaw, 1964).

Assessing the impact of environmental fluctuations on genotype performance is essential to identify genotypes with minimal genotype × environment interaction. Phenotypically stable genotypes with wide adaptability are particularly important for consistent crop performance, as environmental conditions can vary substantially between seasons and years. In the context of ornamental kale, developing genotypes with stable performance across variable environments is crucial for its successful cultivation. Currently, limited research is available on the phenotypic stability of completely homozygous doubled haploid lines in ornamental kale. This study was therefore undertaken to evaluate the stability of DH lines and to investigate whether complete homozygosity and homogeneity restrict the application of haploid techniques in breeding programs. The findings of this investigation are presented in this paper.

Materials and Methods

The experimental material for this study comprised 13 double-haploid (DH) lines of ornamental kale and two commercial check varieties. The evaluation was conducted during the winter seasons of 2021–2023 at the Sarsai farm of ICAR-IARI Regional Station, Katrain (latitude 32°8′07′′ N, longitude 77°10′43′′ E, altitude 1710 m above mean sea level) in Kullu district, Himachal Pradesh, a temperate hill zone in the north-western Himalayas. The experiment followed a randomized block design (RBD) with three replications. Seedlings were transplanted at a spacing of 45 cm × 45 cm during the last week of September each year. Phenotypic observations were recorded for five randomly selected plants from each line within each replication. The traits evaluated included plant height (cm), plant spread (cm), diameter of the central-coloured portion (cm), stem thickness (mm), average head size (cm), days to head formation and days to colour initiation.

Stability parameters were estimated to assess genotype-environment (G × E) interactions. These included the mean performance of the trait $\left(\overline{X}\right)$, the linear regression coefficient (bi) and the mean square deviation from regression (S^2di). The mean trait value $\left(\overline{X}\right)$ reflects the overall performance of a genotype, while *bi* and S^2di capture the G × E interaction. A non-significant G × E interaction, particularly when the linear component predominates over the non-linear component, allows for more reliable stability predictions. According to the stability model proposed by Eberhart and Russell (1966), the S^2di parameter measures the predictability of performance,

Table : Qualitative traits of Doubled haploid (DH) lines of ornamental kale.

Genotype	Colour of leaf margins	Inner leaf colour	Leaf pattern	Potential uses
KtDH-29	Green	Pink	Cabbage	Cut
KtDH-30	Green	Purple green	Wavy	Cut
KtDH-26	Green	Purple green	Wavy	Cut
KtDH-27	Green	Pink	Wavy	Cut
KtDH-56	Green	Pink	Wavy	Bedding
KtDH-13	Green	Purple	Wavy	Bedding
KtDH-52	Purple	Light yellow	Wavy	Bedding
KtDH-10	Green	Purple	Wavy	Cut
KtDH-55	Green	Pink	Fringed	Bedding
KtDH-57	Green	White pink	Cabbage	Cut
KtDH-37	Purple green	Violet pink	Wavy	Bedding
KtDH-45	Green	Yellow green	Wavy	Cut
KtDH-19	Purple green	Purple	Fringed	Bedding
Crane red	Purple	Dark purple	Cabbage	Cut
Nagoya Mix	Green	White /purple	fringed	Bedding

while *bi* quantifies stability. An ideal genotype exhibits high mean performance, a *bi* value close to unity, and minimal deviation from regression ($S^2di \approx 0$). Based on this model, genotypes are classified as follows: (i) below-average stability, performing well only under favourable conditions (*bi* >1); (ii) above-average stability, adapted to less favourable environments (*bi* < 1); and (iii) average stability, demonstrating consistent performance across diverse environments (*bi* =1). The three winter seasons (2021, 2022 and 2023) were treated as distinct environments for the stability analysis. Data were analyzed for stability parameters using the method of Eberhart and Russell (1966), with computations performed using *OPSTAT* software (Sheoran *et al*., 1998).

Results and Discussion

The analysis of variance (Table 1) demonstrated significant differences among genotypes and across environments for all traits studied, indicating substantial diversity. Specifically, plant height and the diameter of the central-coloured portion varied significantly across environments. The genotype × environment (G×E) interactions were significant for plant spread and average head size, highlighting the differential response of genotypes under varying environmental conditions. These findings are consistent with the results reported by Khan *et al*. (2008) in kale. Significant mean squares for the environmental (linear) component, except for stem thickness, average head size and days to head formation, revealed notable environmental variations over the three years of study. This variation was attributed to differing environmental conditions during the study period. The linear component of the G×E interaction was significant for plant spread and days to head formation, indicating that linear regression accounted for most of the stability differences, allowing for reliable performance predictions for these traits under varying environments. For traits exhibiting unpredictable responses, individual genotype stability parameters must be considered. Similar observations were reported by Sharma *et al*. (2006) in cabbage.

Following the Eberhart and Russell model (1966), 15 genotypes were assessed for stability parameters, including the mean performance, regression coefficient (bi) and deviation from regression (S^2di), to classify them based on their stability and adaptability across environments (Tables 2a and 2b). For plant height, genotypes KtDH-29, KtDH-27, KtDH-10 and Crane Red exhibited high mean values, regression coefficients greater than unity (bi > 1) and non-significant

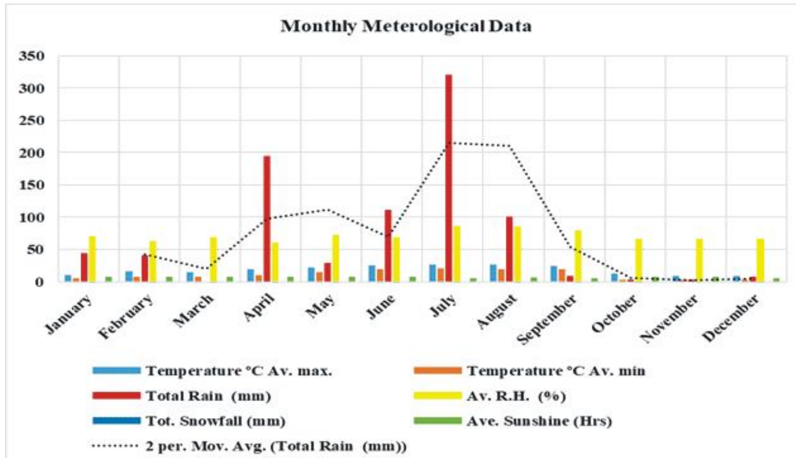

Fig. 1 : Metrological data during January 2021–December 2021.

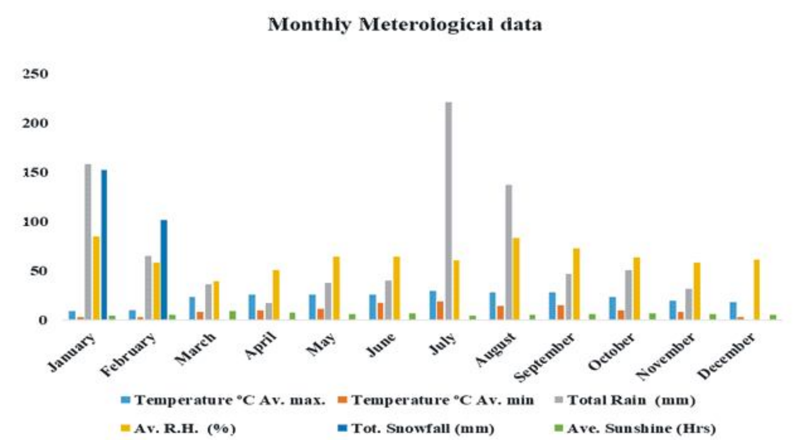

Fig. 2 : Metrological data during January 2022–December 2022.

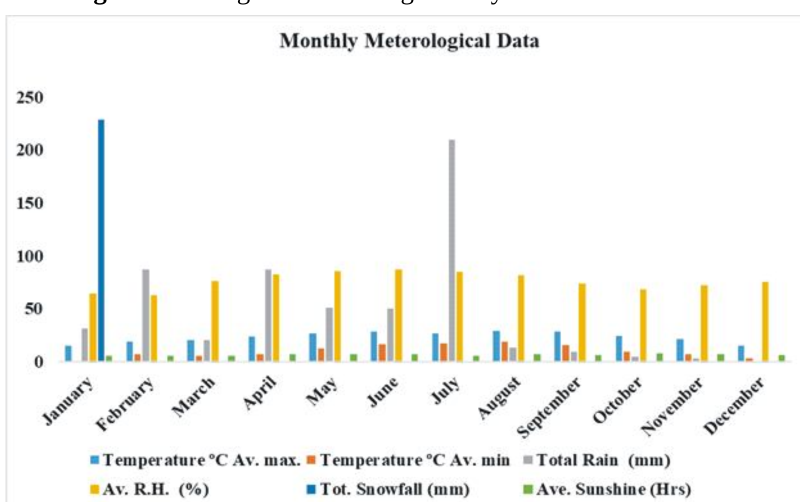

Fig. 3 : Metrological data during January 2023–December 2023.

Table 1 : Pooled analysis of variance (mean squares) for different traits in DH lines of ornamental kale.

Source of variation	df	Plant height (cm)	Plant spread (cm)	Diameter of central coloured portion (cm)	Stem thickness (mm)	Average head size (cm)	Days to head formation (days)	Days to colour initiation (days)
Genotype	14	234.795**	161.389**	18.305**	46.726**	116.748**	225.840**	245.922**
Environment	2	4.692**	1.538	16.348**	0.753	0.652	0.096	1.156
Genotype x Environment	28	0.734	2.372**	0.998	0.409	4.152**	2.975	0.769
Environment+ (Genotype x environment)	30	0.998	2.316	2.021	0.432	3.918**	2.783	0.795
Environment (Linear)	1	9.384**	3.077**	32.696**	1.506	1.303	0.193	2.311**
Genotype x environment (linear)	14	0.818	2.109**	1.892	0.671	0.173	4.672**	1.281
Pooled deviation	15	0.606	2.459**	0.096	0.138	7.588**	1.192	0.240
Pooled Error	84	1.737	2.992*	0.391	1.254	2.011	3.767**	3.219**

*, ** Significant at P = 0.05 and P = 0.01, respectively.

Table 2a : Estimation of stability parameters for plant height, plant spread and diameter of central coloured portion in 13 DH lines of ornamental kale with commercial checks.

Genotype	Plant height (cm)			Plant spread (cm)			Diameter of central coloured portion (cm)		
	Mean	bi	S^2di	Mean	bi	S^2di	Mean	bi	S^2di
KtDH-29	30.156	1.528	-0.203	16.183	-2.751	-0.994	9.539	1.373**	-0.127
KtDH-30	29.211	2.338*	-0.261	17.212	2.693	4.296	7.711	1.409**	-0.126
KtDH-26	32.556	3.851**	4.043**	17.744	0.452	0.039	9.233	0.680**	-0.122
KtDH-27	28.656	1.169	-0.421	22.582	-1.400	-0.786	8.089	0.083	0.080
KtDH-56	17.756	0.168	0.915	40.300	-4.285	1.866	9.350	0.058	-0.103
KtDH-13	19.200	0.144	-0.263	26.978	1.189	-0.991	9.389	0.365	-0.124
KtDH-52	17.033	1.002	-0.541	35.629	-1.381	4.336	11.714	1.973**	-0.004
KtDH-10	28.667	1.002	-0.541	26.194	4.776	8.692*	10.268	2.458**	-0.030
KtDH-55	15.894	0.334	-0.575	32.436	1.784	3.073	10.881	1.943**	-0.028
KtDH-57	49.961	-0.668	-0.562	25.667	-2.192	-0.927	11.739	2.495**	-0.039
KtDH-37	21.632	-0.334	-0.575	31.550	-0.461	-0.527	5.483	0.343	0.280**
KtDH-45	26.778	1.336	-0.511	28.456	0.746	-0.815	7.750	-0.142	0.030
KtDH-19	20.500	0.056	0.868	32.133	5.845	1.092	14.773	0.238	0.000
Crane red	29.311	1.737	-0.464	29.400	3.456	-0.907	8.333	0.133	-0.086
Nagoya Mix	16.978	1.336	-0.511	37.583	6.530	4.480	14.156	1.592**	-0.119
Pooled mean	25.62			28.00			9.89		
S.E. (mean) ±	0.55			1.11			0.22		
S.E. (b) ±	0.98			3.46			0.21		

bi: Regression coefficient, S^2di: Squared deviation from regression coefficient: *, ** Significant at P =0.05 and P =0.01, respectively.

S^2di values, demonstrating stability and suitability for favorable environments. Conversely, KtDH-57 showed a high mean value, bi < 1 and non-significant S^2di, making it stable and suitable for unfavourable environments. KtDH-26, despite its high mean plant height, exhibited instability due to significant regression and deviation. Genotypes KtDH-52 and KtDH-10, with bi ≈ 1, were broadly adaptable, while KtDH-19 (Xi < 25.62, bi < 1, and lower S^2di) showed stability under poor environments, aligning with the findings of Khan *et al*. (2008) in kale.

For plant spread, genotypes KtDH-56, KtDH-52, KtDH-55, KtDH-37, KtDH-19, Nagoya Mix, Crane Red

Table 2b : Estimation of stability parameters for stem thickness, average head size and days to head formation in 13 DH lines of ornamental kale with commercial check.

Genotype	Stem thickness (mm)			Average head size (cm)			Days o head formation (days)			Days to colour initiation (days)		
	Mean	bi	S²di	Mean	bi	S²di	Mean	bi	S²di	Mean	bi	S²di
KtDH-29	20.586	-1.016	-0.015	12.911	0.811	6.298	72.111	1.154	1.023	49.333	1.731	-0.646**
KtDH-30	19.450	-1.697	-0.185	12.822	1.614	11.748	61.556	-16.154	0.801	33.778	4.135**	-0.966**
KtDH-26	15.818	3.061**	-0.401**	12.994	0.814	11.275	54.778	-14.423	-1.186	31.000	0.865	-0.966**
KtDH-27	14.953	5.855**	-0.185	13.700	2.397	-0.430	72.444	27.115**	0.489	53.444	-3.942	-0.727**
KtDH-56	11.402	3.855**	-0.282*	12.006	2.002	-0.111	50.667	-21.346*	-0.217	41.111	-0.192	-1.005**
KtDH-13	11.964	0.690	-0.390**	11.267	1.610	5.733	64.889	22.500*	-1.015	43.778	1.827	-1.069**
KtDH-52	13.389	1.982	-0.345*	27.141	-1.628	46.101**	74.333	25.385**	0.026	60.556	2.212	-0.864**
KtDH-10	13.576	2.553*	-0.383**	17.994	0.809	4.871	68.111	-19.615**	-0.566	32.000	1.154	-1.056**
KtDH-55	13.631	2.270	-0.417**	21.556	0.785	11.279	75.778	34.615**	6.545**	35.444	7.596**	-0.556*
KtDH-57	24.297	-0.081	0.145	24.533	1.599	-0.664	45.889	-17.885	-0.844	31.000	-0.577	-0.458
KtDH-37	13.014	2.270	-0.417**	26.573	1.007	-0.641	59.000	-15.000	-1.256	40.222	1.058	-0.727**
KtDH-45	18.911	-2.520	-0.092	27.200	2.397	-0.563	67.778	-3.462	-1.113	47.667	-3.462**	-0.919**
KtDH-19	22.611	-2.829*	-0.408**	20.109	-1.611	6.913	61.778	5.769	-1.165	31.222	3.365**	-0.966**
Crane red	15.650	2.574*	-0.413**	15.689	-0.798	-0.456	66.000	-4.038	-1.243	38.556	-1.538	-0.919**
Nagoya Mix	15.931	-1.967	-0.418**	26.467	3.191	2.415	60.889	10.385	-1.233	35.556	0.769	-0.646**
Pooled mean	16.35			18.86			63.73			40.31		
S.E. (mean) ±	0.26			1.95			0.77			0.35		
S.E. (bi) ±	1.17			9.35			9.64			1.25		

bi: Regression coefficient, S²di: Squared deviation from regression coefficient: * , ** Significant at P =0.05 and P =0.01, respectively.

and KtDH-45 exhibited higher mean values. Genotypes such as KtDH-55 and KtDH-19, with bi > 1, were suited to favorable environments, while KtDH-56, KtDH-52, and others with bi < 1 were suitable for unfavorable conditions. KtDH-29, which displayed minimal plant spread with non-significant bi and S²di, indicated average stability and adaptability to favorable conditions. A compact plant spread is often desirable for ornamental crops, as noted by Ram (1992). Similar trends were observed by Chaubey *et al*. (2000) in cabbage.

Regarding the diameter of the central-coloured portion, KtDH-19 showed stability and suitability for unfavourable environments due to its high mean value and non-significant bi ≈ 1. For stem thickness, KtDH-57 was identified as suitable for both favourable and unfavourable environments due to its high mean value, bi < 1, and non-significant S²di, indicating predictable performance. The stability analysis for average head size (Table 2b) revealed variability among genotypes. KtDH-37, with a higher-than-average head size, bi ≈ 1 and S²di = 0, exhibited wide adaptability, making it a valuable candidate for breeding programs. KtDH-57, with bi > 1 and S²di = 0, was stable for favourable environments, while KtDH-52, showing significant S²di was deemed unstable for this trait.

For days to head formation, KtDH-57 emerged as a desirable early-maturing genotype due to its low mean value, bi ≈ 1 and S²di = 0, indicating stability. Other genotypes, such as KtDH-27 and Crane Red, exhibited above-average mean performance but were unstable due to significant deviations. Early maturing genotypes are preferred for this trait, in line with Chaubey *et al*. (1999). With regards to days to colour initiation, all genotypes except KtDH-57 showed significant S²di values, reflecting instability across environments. Early coloration is desirable for ornamental kale, enhancing its aesthetic appeal. Based on the Eberhart and Russell stability criteria, KtDH-57 was identified as the most stable and desirable genotype for days to colour initiation.

Conclusion

The genotypes KtDH-57 and KtDH-19 demonstrated consistent and predictable performance across a majority of traits studied, making them ideal candidates for commercial cultivation in mid-hill temperate regions of Himachal Pradesh. This study underscores the utility of double haploid lines in ornamental kale breeding programs, highlighting that their homozygosity and homogeneity do not constrain their effectiveness.

References

Allard, R.W. and Bradshaw A.D. (1964). Implications of genotype-environmental interactions in applied plant breeding. *Crop Sci*., **4**, 503-508.

Allard, R.W. (1960). *Principles of Plant Breeding*. John Willey and Sons Inc., New York.

Chaubey, T., Srivastava B.K. and Singh M. (1999). Stability analysis for maturity and vegetative characters in cabbage. *Veg. Sci*., **26**, 115-119.

Chauby, T., Shivastava B.K. and Singh M. (2000). Stability analysis of yield and quality contributing characters in cabbage. *Veg. Sci*., **27**, 45-50.

Dunwell, J.M. (2010). Haploids in flowering plants: Origins and exploitation. *Plant Biotechnol. J*., **8**, 377–424.

Eberhart, S.A. and Russel W.A. (1966). Stability parameters for comparing the varieties. *Crop Sci*., **6**, 36-40.

Khan, S.H., Ahmed N., Jabeen N., Wani K.P. and Hussain K. (2008). Stability analysis for economic traits in kale (*Brassica oleracea* var. *acephala* L.). *Indian J. Genet*., **68(2)**, 177-182.

Li, Y. and Yu X. (2006). Pollination with laser-irradiated pollens breaks cross-incompatibility between zicaitai (*Brassica campestris* var. *purpurea*) and ornamental kale (*Brassica oleracea* var. *acephala*) to produce hybrids with the aid of ovule culture. *Sci. Hortic*., **108**, 397–402.

Park, S.J., Walsh E.J., Reinbergs E., Song L.S.P. and Kasha K.J. (1976). Field performance of doubled haploid barley lines in comparison with lines developed by the pedigree and single seed descent methods. *Can. J. Plant Sci*., **56**, 467-474.

Prigge, V., Sánchez C., Dhillon B.S., Schipprack W., Araus J.L. *et al*. (2011). Doubled haploids in tropical maize. I. Effects of inducers and source germplasm on *in vivo* haploid induction rates. *Crop Sci*., **51**, 1498–1506.

Ram, H.H. (1992). *Vegetable breeding: Principles and Practices*. Kalyani Publishers, Ludhiana, pp. 274-289.

Sharma, A., Pathania N.K., Pathak S. and Singh Y. (2006). Stability analysis for marketable head yield and its component horticultural traits in cabbage (*Brassica oleracea* var. *capitata* L.) under dry temperate conditions of north-western Himalayas. *Indian J. Genet*., **66(2)**, 163-164.

Sheoran, O.P., Tonk D.S., Kaushik L.S., Hasija R.C. and Pannu R.S. (1998). Statistical software package for agricultural research workers. In: Department of mathematics statistics, Recent Advances in information theory, Statistics & Computer Applications edited by DS Hooda and RC Hasija, Hisar, India. Chaudhary Charan Singh Haryana Agricultural University, 139-143. http://14.139.232.166/ops tat/default.asp.