

Assessment of Breeding Potential of Horse Gram [*Macrotyloma uniflorum* (Lam.) verdc.] Crosses based on Transgressive Segregation Index and Usefulness Criterion

B. R. CHANDANA AND S. RAMESH

Department of Genetics and Plant Breeding, College of Agriculture, UAS, GKVK, Bengaluru - 560 065

e-Mail : chandanargowda6@gmail.com

ABSTRACT

Being predominantly a self-pollinated crop, pure-lines are the only cultivar options in horse gram. Pedigree selection of desirable recombinant inbred lines (RILs) from segregating populations for use as pure-line cultivars is the most widely used breeding method in horse gram. Elimination of poor crosses in the early generations help efficient utilization of resources for maximizing chances of deriving desirable RILs from a few crosses with high breeding potential. Breeding potential of two $F_{2,3}$ populations [(HPKM 320 × CRIDA 18-R) and (IC 361290 × Palem 1)] derived from parents differing in growth habit and grain yield was assessed based on traits' mean, absolute range (AR), standardized range (SR), phenotypic coefficient of variation (PCV), transgressive segregation index (TSI) and usefulness criterion (Uc), which combines, mean, phenotypic standard deviation and standardized selection differential at different selection intensities. The cross with high quantitative traits' mean, AR, SR, PCV, TSI and Uc in $F_{2,3}$ generations was considered as that with better breeding potential. When descriptive statistics were considered individually HPKM 320 × CRIDA 18-R was found to have better breeding potential than IC 361290 × Palem 1 for grain weight plant⁻¹. Based on Uc, breeding potential of (IC 361290 × Palem 1) $F_{2,3}$ population was better than that of (HPKM 320 × CRIDA 18-R) $F_{2,3}$ population for grain weight plant⁻¹. Based on the results and those of other researchers, Uc could be used as an objective tool for selecting the segregating populations with good breeding potential in horse gram and other self pollinated crops.

Keywords : Breeding potential, Usefulness criterion, Transgressive segregation index, Phenotypic coefficient of variation, Genotypic coefficient of variation

HORSE gram is one of the traditional grain legume crops extensively grown in India. It is the fifth most widely grown grain legume in India (Fuller and Murphy, 2018). It is grown predominantly in rainfed ecosystems. It is known for its resilience to adverse climatic conditions such as drought, salinity and heavy metal stresses (Reddy *et al.*, 2005). It can also be grown in nutrient-poor soils (Yasin *et al.*, 2014 and Bhartiya *et al.*, 2015). It has higher calcium content (289 mg 100 g⁻¹ seed) than any other legume except rice bean (302 mg 100 g⁻¹ seed) (Gopalan *et al.*, 1989). Horse gram is also rich in phosphorus, iron and vitamins such as carotene, thiamine, riboflavin, niacin and vitamin C (Sodani *et al.*, 2006). It supports livelihoods of millions of smallholder rural farming communities in traditional subsistence farming systems and significantly contributes to food and nutritional security

particularly, during dry seasons, drought and famine (Magbagbeola *et al.*, 2010). Besides this, it serves as a valuable crop component to address present and future environmental challenges to agricultural production (Kahane *et al.*, 2013).

Horse gram productivity in India (0.4 t ha⁻¹) at large and Karnataka (0.13 t ha⁻¹) in particular (Anonymous, 2017), is far less than its potential (0.9 t ha⁻¹) realized in research stations (Purushottam *et al.*, 2017). Despite its multiple uses and resilience to harsh climatic conditions, the efforts to breed horse gram to enhance its productivity and economic utility compared to other major legumes such as chickpea, soybean, pigeonpea, cowpea, etc., is rather limited (Fuller and Murphy, 2018). As a result, horse gram producing farmers are constrained by limited cultivar choice and

non-availability of seeds of even a few recommended varieties (Virk *et al.*, 2006). Providing a greater cultivars choice to farmers (among others) helps maximize horse gram productivity by using the most appropriate cultivar for each production environment (Virk *et al.*, 2006).

Being predominantly a self-pollinated crop, pure-lines are the only cultivar options in horse gram. Pedigree selection of desirable recombinant inbred lines (RILs) for use as pure-line cultivars is the most widely used breeding method in horse gram (Ashwini *et al.*, 2021). Very often, a crop breeder is confronted with the task of selecting a few among a large number of bi-parental/multi-parental crosses-derived segregating (breeding) populations to implement pedigree selection to identify superior/ transgressive RILs for use as pure-line cultivars. Elimination of poor crosses in the early generations help efficient utilization of resources for maximizing chances of deriving desirable RILs from large-sized segregating populations derived from a few crosses with good breeding potential (Ramesh and Byregowda, 2016; Suresh *et al.*, 2017; Bernardo, 2020 and Anilkumar *et al.*, 2021).

First-degree statistics such as mean, absolute range (AR) and standardized range (SR) and second-degree statistics such as absolute variance and standardized variance [phenotypic coefficient of variation (PCV)] are being used by breeders to choose best ones from among a large number of segregating populations routinely developed in crop breeding programs (Shivakumar *et al.*, 2016, Suresh *et al.*, 2017; Bernardo 2020 and Anilkumar *et al.*, 2021). An ideal segregating population is the one which has a high mean and a large variance for trait(s) of interest. Starting out with high mean helps ensure that selected progeny will have high mean even when selection is imperfect (Bernardo, 2020). Having a large genetic variance provides opportunities for selection of genotypes with desired combination of traits. Having a relatively high mean and a large genetic variance allows maximum performance of selected progeny (Bernardo, 2020). Taken individually, these statistics lead to different choices of segregating populations. The use of usefulness criterion (Uc)

(Melchinger, 1987), a summary statistic which combines mean, variance and heritability and Transgressive segregation index (TSI) (Koide *et al.*, 2019), which takes into account 'AR' in segregating population in relation to parental AR, provide an objective means to select segregating populations with better breeding potential. The objective of the present study is to identify potential crosses which are likely to result in high frequency of superior RILs in advanced generations based on first and second - degree descriptive statistics, Uc and TSI estimable in their early segregating generations.

MATERIAL AND METHODS

The basic genetic material consisted of a random sample of 39 and 25 $F_{2:3}$ families derived from two crosses namely, HPKM 320 (determinate) \times CRIDA 18-R (indeterminate) and IC 361290 (determinate) \times Palem 1 (indeterminate), respectively. The seeds of four parents and the two check varieties, namely PHG 9 and BGM 1 and randomly selected 39 and 25 $F_{2:3}$ families were planted in a single row of 3m length in randomized complete block design with two replications during 2020 rainy season. Fifteen-days after planting, seedlings of four parents, two check varieties and $F_{2:3}$ families were thinned to maintain a spacing of 0.2m between the plants and 0.3m between the rows. The entire recommended production package was practiced to raise four parents, two check varieties and two $F_{2:3}$ populations. A total 12 plants in each of the four parents, two checks and within each $F_{2:3}$ families were maintained till maturity.

Sampling of Plants and Data Recording

Data were recorded on 10 randomly selected plants (avoiding border ones) in four parents, two check varieties and $F_{2:3}$ progenies in each of the two replications for four traits, namely, number of primary branches and pods and weights of sun-dried pods and grains. The average of these traits across 10 sample plants in each replication was computed and expressed as primary branches plant⁻¹, pods plant⁻¹, pod weight plant⁻¹ (g) and grain weight plant⁻¹ (g).

Estimation of Descriptive Statistics and Quantitative Genetic Parameters

Data recorded on replication-wise mean data of 10 randomly selected plants of four parents, two check varieties and $F_{2,3}$ plants (within each of F_3 progenies) were used to calculate descriptive first-degree statistics such as mean, absolute range (AR) (highest-lowest) and standardized range (SR) [(highest - lowest)/mean] and second degree statistics such as absolute phenotypic variance σ_p^2 , phenotypic standard deviation (σ_p), standardized phenotypic variance, *i.e.*, phenotypic coefficient of variance (PCV) = $[(\sigma_p/\text{mean}) \times 100]$. The significance of trait means and σ_p^2 between the two crosses was examined using two-sample 't' test and Levene's test (Levene, 1960), respectively implemented using statistical option available in Microsoft Excel software. Transgressive segregation index (TSI) was estimated as (AR/trait mean difference between parents). The additive genetic variance (σ_A^2) was estimated by equating observed and expected mean squares (MS) due to 'between $F_{2,3}$ families' from analysis of variance (ANOVA) of $F_{2,3}$ families and solving for σ_A^2 (Ooijen 1989) as $\sigma_A^2 = 2 \times [(MS \text{ due to 'between } F_{2,3} \text{ families' - MS due to error}) / (\text{number of replications})]$. MS attributable to residual variance from ANOVA was added to σ_A^2 to estimate phenotypic variance (σ_p^2). Genotypic coefficient of variance (GCV) was estimated as $GCV = [(\sigma_g/\text{mean}) \times 100]$. The σ_g was estimated as square root of σ_g^2 . The σ_g^2 was estimated as $\sigma_p^2 - \sigma_e^2$, where, σ_e^2 is MS due to error. Usefulness criterion (Uc) for each trait was estimated as trait mean + $(k \times \sigma_A^2 / \sigma_p)$, where, k = standardized selection differential at different selection intensities; k = 2.67, 2.06, 1.76, 1.55, 1.40, 1.27 and 1.16 at 1, 5, 10, 15, 20, 25 and 30 per cent selection intensities, respectively (Bernardo, 2020).

Criteria to Assess the Breeding Potential of Crosses

The cross with high quantitative traits' mean, AR, SR, PCV, TSI and Uc in $F_{2,3}$ generations was considered as those with better breeding potential.

RESULTS AND DISCUSSION

Prioritizing the limited resources (among others) is the key to success in breeding crops with no exception of horse gram. Crop breeders face the challenge of selecting breeding populations with good breeding potential in terms of recovery of desirable RILs for use as pure-line cultivars (Witcombe and Virk, 2001). In this article, we provide results to demonstrate the utility of simple descriptive statistics such as mean, range and variance considered individually and of their combination represented by TSI and Uc for choosing the cross with good breeding potential in horse gram. Significance of mean sum of squares attributable to $F_{2,3}$ families as indicated by ANOVA (Table 1) suggest substantial variation among genotypes of $F_{2,3}$ generation. This variation could be attributed to a large difference between their parents at several loci controlling all the four traits under study. Significant large variation among $F_{2,3}$ families justifies the assessment of breeding potential of the two crosses.

Mean and Range

Both mean and range are widely used descriptive statistics to summarize observed pattern of variation in target traits as they are simple to estimate and intuitive for interpretation. Having a high mean and range of target trait(s) provide a head start for selecting desirable genotypes from segregating populations. Based on trait means, the results indicate that HPKM 320 \times CRIDA 18-R is better than IC 361290 \times Palem 1 for pods plant⁻¹ and pod weight plant⁻¹; the two crosses are comparable for other two traits (Fig. 1). However, HPKM 320 \times CRIDA 18-R is better than IC 361290 \times Palem 1 for all the four traits with respect to AR (Table 2). On the contrary, IC 361290 \times Palem 1 is better than HPKM 320 \times CRIDA 18-R in terms of unit-independent estimates of SR for primary branches plant⁻¹, pods plant⁻¹ and pod weight plant⁻¹; while the later cross is better than the former one with respect to grain weight plant⁻¹.

TABLE 1
Analysis of variance of F_{2:3} progeny families for four quantitative traits in horse gram

Source of variation	Degrees of freedom		Primary branches plant ⁻¹		Pods plant ⁻¹		Pod weight plant ⁻¹ (g)		Grain weight plant ⁻¹ (g)	
	HPKM 320 × CRIDA 18-R	IC 361290 × Palem 1	HPKM 320 × CRIDA 18-R	IC 361290 × Palem 1	HPKM 320 × CRIDA 18-R	IC 361290 × Palem 1	HPKM 320 × CRIDA 18-R	IC 361290 × Palem 1	HPKM 320 × CRIDA 18-R	IC 361290 × Palem 1
Between F _{2:3} families	38	24	1.37 **	0.44	72.98 **	79.06 **	3.63 **	2.21 *	1.30 **	1.27 **
Replication	01	01	0.01	0.36	20.99	1.14	6.34	0.19	1.15	0.12
Error	38	24	0.27	0.32	19.87	16.84	1.39	0.5	0.55	0.31

*Significant at P=0.05 **Significant at P=0.01

The Transgressive Segregation Index (TSI)

Without transgressive segregation (TS), plant breeding does not work; plant breeding does work, therefore TS occurs (Mackay *et al.*, 2020). While HPKM 320 × CRIDA 18-R displayed higher TSI than IC 361290 × Palem 1 for primary branches plant⁻¹ and yield of marketable and consumable product (grain weight plant⁻¹); the reverse is true for pods plant⁻¹ and yield of harvestable product (pod weight plant⁻¹) (Table 2). Jambormias *et al.* (2015) in mung bean, Shreya *et al.* (2017) in groundnut, Suresh *et al.* (2017) in dolichos bean and Guindon *et al.* (2018) in pea have also reported TS for economically important traits. Theoretical investigations have indicated that TS results from dispersion of favorable complementary alleles between the parents from which segregating population are derived (Bernardo, 2020 and Mackay *et al.*, 2020). These theoretical studies suggests that alleles that increase primary branches plant⁻¹ and grain weight plant⁻¹ are dispersed between HPKM 320 and CRIDA 18-R, while those that increase pods plant⁻¹ and pod weight plant⁻¹ are dispersed between IC 361290 and Palem 1.

Absolute and Standardized Measures of Phenotypic and Genotypic Variation

In addition to means, it is important to quantify the variability for target traits to arrive at a more informed decision regarding selection of segregating populations with better breeding potential (Tabanao and Bernardo, 2005; Allier *et al.*, 2019; Lehermeier *et al.*, 2019; Bernardo, 2020; Millicent *et al.*, 2020 and Ongom *et al.*, 2021). Crop breeders target highly variable segregating populations as they create opportunities for selection and genetic improvement (Ongom *et al.*, 2021). The estimates of PCV represent true reflection of variability unlike AR and SR which are biased by extreme values. In the present study, both the crosses were comparable with respect to σ_p^2 for all the traits except pod weight plant⁻¹, for which HPKM 320 × CRIDA 18-R was more variable than IC 361290 × Palem 1 (Table 3). The cross, IC 361290 × Palem 1 was also more variable than HPKM 320 × CRIDA

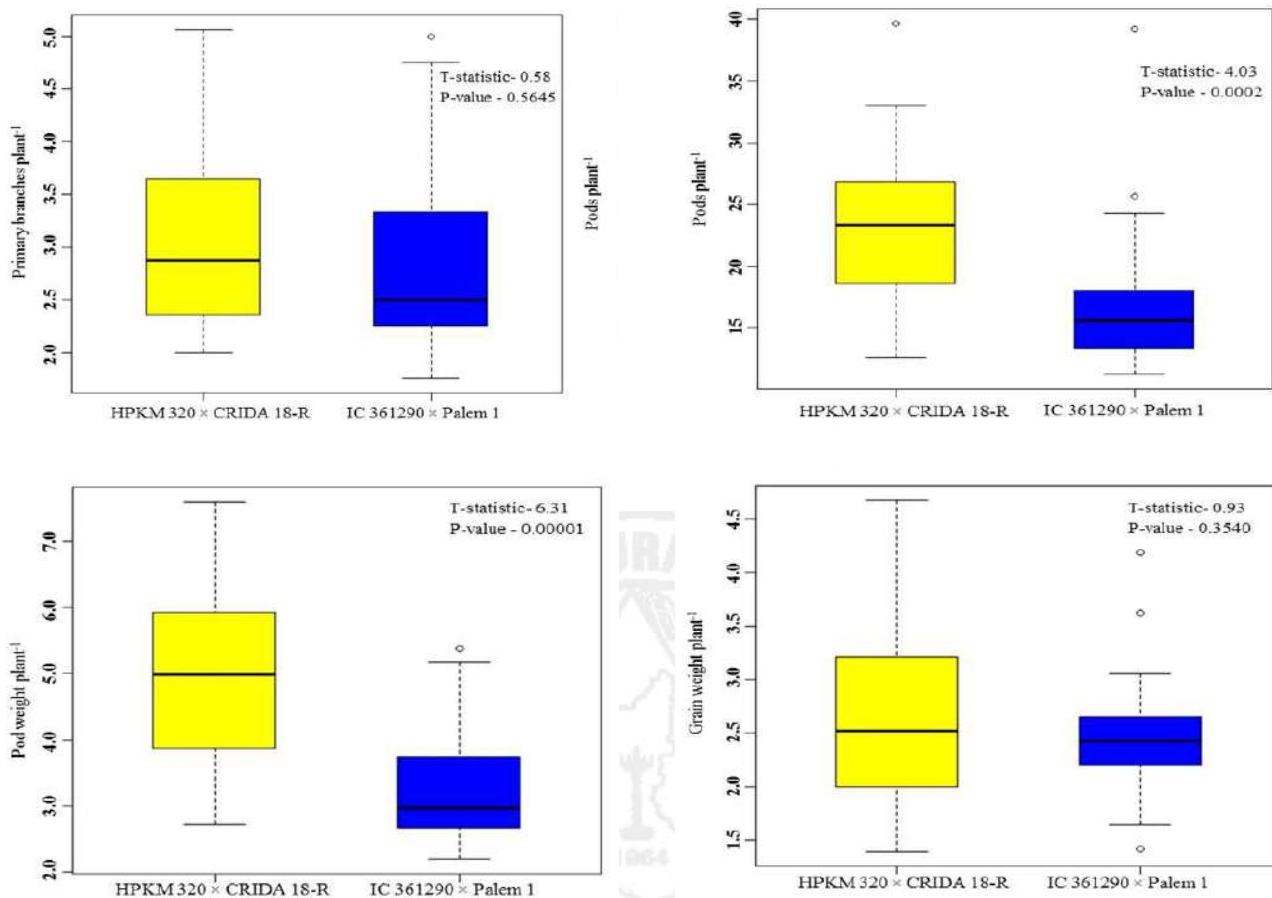


Fig. 1:Box-Whisker plots showing significance of differences in trait means of four quantitative traits in two crosses of horse gram

TABLE 2
Estimates of absolute range, standardized range and transgression index in F_{2,3} generations derived from two crosses in horse gram

Trait	Absolute range (Highest - lowest)		Standardized range [(Highest - lowest)/mean]		Transgressive segregation index (TSI)	
	HPKM 320 x CRIDA 18-R	IC 361290 x Palem 1	HPKM 320 x CRIDA 18-R	IC 361290 x Palem 1	HPKM 320 x CRIDA 18-R	IC 361290 x Palem 1
Primary branches plant ⁻¹	3.33	3.00	1.09	1.21	1.53	1.24
Pods plant ⁻¹	32.83	31.20	1.41	1.83	1.07	5.51
Pod weight plant ⁻¹ (g)	6.23	4.40	1.25	1.34	1.56	4.25
Grain weight plant ⁻¹ (g)	4.09	2.96	1.56	1.21	4.89	4.40

18-R for pods plant⁻¹ and pod weight plant⁻¹ with respect to unit-independent and standardized variability measure (σ_p^2), *i.e.*, PCV; the latter cross was more variable than the former one for other two traits, namely primary branches plant⁻¹ and grain weight plant⁻¹ (Table 3). With respect to another unit-independent and standardized variability measure attributable to genotype, *i.e.*, GCV, IC 361290 × Palem 1 was more variable than HPKM 320 × CRIDA 18-R for all the traits except primary branches plant⁻¹ (Table 3). This cross is expected to respond well to selection and to have better breeding potential in terms of recovery of superior RILs. The differences between F_{2,3} generations derived from the two crosses for PCV could be attributed to differences in the number of favorable genes for which the parents of the two crosses differ (Kearsey & Pooni, 1996 and Bernardo, 2020). These inferences are further supported by theoretical results which indicate that crosses whose parents differ for a larger number of favorable genes would exhibit higher traits means and variances than those whose parents differ for fewer genes (Kearsey and Pooni, 1996; Bernardo, 2020 and Mackay *et al.*, 2020).

The estimates of σ_A^2 were higher in IC 361290 × Palem 1 than those in HPKM 320 × CRIDA 18-R for pods plant⁻¹ and grain weight plant⁻¹; they were higher in HPKM 320 × CRIDA 18-R than those in IC 361290 × Palem 1 for other two traits (Table 4). Similarly the

estimates Considering that σ_A^2 , which is a fixable component of trait variation and chief cause for the resemblance between parent and offspring (Kearsey and Pooni, 1996 and Bernardo, 2020), the results suggest that selection would be more effective and results in a greater genetic gain from IC 361290 × Palem 1 than from HPKM 320 × CRIDA 18-R for yield of marketable and consumable product, *i.e.*, grain. From the preceding results and discussion, it follows that the ranking of the two crosses with respect to breeding potential differ with the statistics and the trait. This necessitates the use of a more comprehensive quantitative measure which combines different first and second degree statistics to assess the breeding potential of crosses. Usefulness criterion (Uc) is one such comprehensive quantitative measure to assess breeding potential of crosses.

Usefulness Criterion (UC)

UC accounts for trait mean, phenotypic variability and narrow-sense heritability and provide a more comprehensive and informative statistic in terms of genetic gain expected when different intensities of selection are imposed in segregating populations (Allier *et al.*, 2019; Lehermeier *et al.*, 2019; Bernardo, 2020; Millicent *et al.*, 2020 and Ongom *et al.*, 2021). A fairly high estimates of additive genetic variance and narrow-sense heritability (Table 4) suggests effectiveness of selection for all the trait under study

TABLE 3

Estimates of absolute and standardized measures of variation in F_{2,3} generations derived from two crosses in horse gram

Traits	Absolute phenotypic variance (σ_p^2)				Phenotypic coefficient of variation (PCV)		Genotypic coefficient of variation (GCV)	
	HPKM 320 × CRIDA 18-R	IC 361290 × Palem 1	Levene statistic	P-value	HPKM 320 × CRIDA 18-R	IC 361290 × Palem 1	HPKM 320 × CRIDA 18-R	IC 361290 × Palem 1
Primary branches plant ⁻¹	1.37	0.44	0.60	0.441	38.25	26.86	34.27	14.02
Podsplant ⁻¹	72.99	79.09	0.519	0.474	36.81	52.25	31.40	46.35
Pod weight plant ⁻¹ (g)	3.49	2.12	9.59	0.003	37.51	44.53	29.10	38.92
Grain weight plant ⁻¹ (g)	1.29	1.27	3.58	0.063	43.35	46.00	32.83	39.99

TABLE 4

Estimates of additive genetic variance and narrow-sense (ns) heritability in F_{2:3} generations derived from two crosses in horse gram

Traits	Additive genetic variance (σ^2_A)		Narrow-sense heritability (h^2_{ns})	
	HPKM 320 × CRIDA 18-R	IC 361290 × Palem 1	HPKM 320 × CRIDA 18-R	IC 361290 × Palem 1
Primary branches plant ⁻¹	1.10	0.12	80.29	27.27
Pods plant ⁻¹	53.12	62.22	72.78	78.67
Pod weight plant ⁻¹ (g)	2.10	1.62	60.17	76.42
Grain weight plant ⁻¹ (g)	0.74	0.96	57.36	75.59

even in early segregating generations. Uc has been suggested as an unambiguous objective method of selecting early segregating generations to increase the prospects of recovering superior RILs for use as cultivars in self-pollinated crops like horse gram. In the present study, HPKM 320 × CRIDA 18-R is more useful than IC 361290 × Palem 1 for primary branches

plant⁻¹, pods plant⁻¹ and yield of harvestable product (podweight plant⁻¹) (Fig. 2). On the other hand, IC 361290 × Palem 1 is more useful than HPKM 320 × CRIDA 18-R for yield of marketable and consumable product (grain weight plant⁻¹) at all selection intensities. When descriptive statistics are considered individually HPKM 320 × CRIDA 18-R is found to have better breeding potential than IC 361290 × Palem 1 for grain weight plant⁻¹.

The findings suggest that (IC 361290 × Palem 1) F_{2:3} population is expected to respond to selection at different intensities better than (HPKM 320 × CRIDA 18-R) F_{2:3} population for the most important trait, i.e., grain weight plant⁻¹. Higher Uc of HPKM 320 × CRIDA 18-R and IC 361290 × Palem 1 could be attributed to greater breeding values of their constituent genotypes for primary branches plant⁻¹, pods plant⁻¹ and pod weight plant⁻¹ and grain weight plant⁻¹, respectively. The chances of recovering desirable RILs are higher from IC 361290 × Palem 1 than those from HPKM 320 × CRIDA 18-R at all selection intensities for grain weight plant⁻¹.

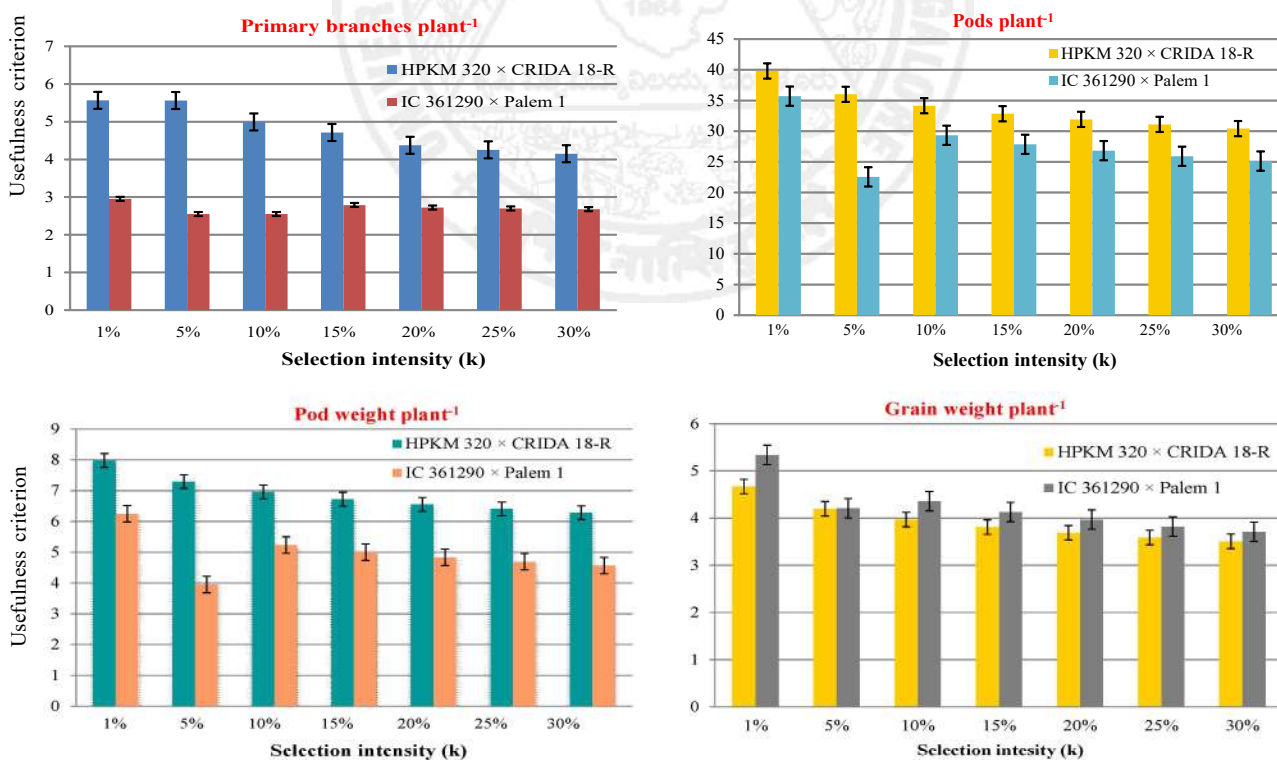


Fig. 2: Bargraphs showing differences in estimates of usefulness criterion for four quantitative traits at different selection differentials (k) in two crosses of horse gram

However, a little relaxed selection intensity (15 to 20%) is expected to avoid elimination of genotypes with stable performance for grain yield (Allier *et al.*, 2019; Lehermeier *et al.*, 2019; Bernardo, 2020; Millicent *et al.*, 2020 and Ongom *et al.*, 2021). 'Uc' has been used to predict the performance of progeny of selected plants from early segregating populations (Allier *et al.*, 2019; Lehermeier *et al.*, 2019; Bernardo, 2020; Millicent *et al.*, 2020 and Ongom *et al.*, 2021). The advantage of 'Uc' is that it captures overall genetic worth of segregating populations. The concept of 'UC' has been extensively used in maize (Tabanao and Bernardo, 2005) and cowpea (Millicent *et al.*, 2020 and Ongom *et al.*, 2021) to identify best breeding populations for extracting superior RILs for use as hybrid parents and cultivars, respectively. Based on our results and those of other researchers (Tabanao and Bernardo, 2005; Millicent *et al.*, 2020 and Ongom *et al.*, 2021), it can be concluded that 'Uc', which takes into account traits 'mean', 'variance' and 'heritability' could be used as objective tool for selecting the segregating populations with good breeding potential to derive superior RILs for use as pure-lines cultivars in other self-pollinated crops.

Acknowledgement: The author gratefully acknowledges the Department of Science and Technology (DST), Government of India for providing financial support in the form of INSPIRE-fellowship DST/INSPIRE Fellowship/IF180603 dated: 25/09/2019 for conducting thesis research for partial fulfillment for the award of Ph.D. degree by the University of Agricultural Sciences, Bangalore, India.

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(Received : July 2021 Accepted : October 2021)