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Independent segregation of qualitative traits and estimation of genetic parameters and gene action for some quantitative traits in guar (*Cyamopsis tetragonoloba* L. Taub.)

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Abstract

Current study was undertaken to elucidate the inheritance qualitative traits namely, flower colour and leaf hairiness in two F_2 populations comprising of 194 and 99 individuals with reference to yield and agronomic traits along with genetic parameters. The violet colour of flowers and leaf hairiness were dominant over white flower and smooth leaves, respectively. The χ^2-test revealed independent single gene inheritance for both the traits with joint segregation ratio of 9:3:3:1 in F_2. Except for no. of pods per cluster and pods per plant, both parents (Smooth 25 and HG2-20) exhibited significant ($p < 0.05$) differences for traits under investigation. A higher estimate of phenotypic coefficient of variance (PCV) than genonotypic coefficient of variance (GCV) was recorded for all the characters except test weight and clusters on branches. The heritability ranged from 24.36% (seeds per pod) to 92.32% (clusters on branches). Gene action as per skewness indicated that both duplicate and complementary interactions were playing role in the development of various traits, whereas, inheritance analysis as per kurtosis suggested that traits were oligogenic. Eventually, it can be concluded that high GCV, appearance of transgressive segregants and involvement of fewer major genes in defining the quantitative traits including yield, provided an opportunity for their genetic improvement.

Keywords: Guar, chi-square, heritability, kurtosis, mapping population, skewness

Introduction

Guar [*Cymopsis tetragonoloba* (L.) Taub] is a versatile crop of arid and semi-arid regions of India and Pakistan (Mahla et al. 2018). Being an industrially important crop it has been introduced into many countries like Australia, Bangladesh, Myanmar, USA, South Africa, Brazil, Congo and Sri Lanka and occupies small areas (Boghra et al. 2016; Kumar et al. 2017).

Every part of this multipurpose crop is useful and valuable. Plant parts like stem, leaves and pod shell are good for fodder, whereas, green pods are used as vegetable. The most valuable part of guar is the seed being a rich source of galactomannan, which accumulates in endosperm. Endosperm constitutes about 30-35% of the whole seed and is the most valuable part due to its industrial value. Realizing its economic importance systematic crop improvement efforts resulted in to release of about 40 varieties in India (Bhatt et al. 2016). Interestingly, all improved varieties of cluster bean grown for seed purpose in India are pubescent (hairy leaf and stem) and violet flowered except one (RGC-936) which is white flowered. Both pubescence (Kinman et al. 1962; Sohoo and Gill 1976; Memon 1980; Chaudhary and Singh 1980) and flower colour (Memon, 1980) exhibit simple Mendelian monogenic inheritance in cluster bean. Presence of pubescence plays a crucial role in plant protection against biotic stresses (Soroka et al. 2011; Alahakoon et al. 2016; Ushan et al. 2016; Cui et al. 2016), but makes harvesting of crop a challenging operation. Further, hairs protect the cells from abiotic stresses like solar radiations, chilling injury and transpiration by increasing surface area of leaf and acting as a cover overleaf surface (Ehrlinger 1984; Skaltsa et al. 1994; Agrawal et al. 2004). An earlier study on leaf trichomes in *Oryza nivara,* indicated that hairiness reduces the water loss under moderate to high solar intensities and eventually improved the photosynthetic

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water use efficiency (Hamaoka et al. 2017). Being a minor legume, such studies establishing yield advantage of hairiness are absent in guar.

Advantage of violet flower color is not directly evident as guar is a self-pollinated crop where flower colour may not help in pollination. However, earlier reports suggested that indirect selection mediated via (a)biotic stresses could maintain flower colour variation in self-pollinated crops (Vaidya et al. 2018). Guar is a crop of arid region and pigmentation in flowers may support the fitness advantage under hot and drought-prone environments. To assess the role of flower colour, Strauss et al. (2004) conducted a study in wild radish and reported a correlation between flower pigment and insect herbivory and concluded that common biosynthetic pathways of both secondary defensive metabolites and flower pigmentation might be responsible for such correlation. Consequently, violet flower colour preferably appeared in the released varieties due to natural consequence enhance the fitness of guar in semi-arid regions of the globe especially India. Therefore, in current investigation, genetics of pubescence and flower pigment was studied to exploit these qualitative traits in future breeding programs for guar improvement.

Understanding the inheritance of monogenic traits with polygenic traits is an important step in plant breeding to augment such traits. This allows efficient incorporation of inherited desirable traits in the well-adapted lines/varieties with higher production potential while offering new distinguishing markers to satisfy DUS (distinctness, uniformity and stability) requirements. However, no information on coinheritance pattern of the two traits is available in guar. Keeping in view the above facts, a study was carried out to get detailed information on genetics and coinheritance of flower colour and leaf hairiness, followed by analysis of genetic components and gene action for various traits in F_2 generation of guar.

Materials and methods

Plant material

For the development of suitable segregating population, two morphologically diverse parents for many traits were selected. Smooth genotypes (Smooth 25 and Smooth 22) were crossed to a hairy parent (HG 2-20). Both female parents Smooth 25 (Fig. 1) and Smooth 22 were white-flowered and smooth foliage and stem, while male parent HG 2-20 (Fig. 2) was a hairy leaf variety having violet flowers. The crosses (Smooth 25 x HG2-20 and Smooth 22 x HG2-20) were made manually and resultant F_1 was selfed to get F_2 population comprising of 194 and 99 segregants, respectively.

Fig. 1. Smooth-25

Fig. 2. HG2-20

Field evaluation

For field evaluation, the segregating progenies were raised at 50 cm row to row and 20 cm plant to plant spacing. Each plant was phenotyped for the presence and absence of pubescence (hairy/smooth) and flower colour (violet/white). Likewise, phenotypic data were recorded on a total of 10 agronomic traits *viz*., plant height (PH; cm), no. of branches per plant (BPP), clusters on main stem (CMS), clusters on branches (CB), pods per cluster (PPC), no. of pods per plant (PPP), pod length (PL; cm), seeds per pod (SPP), yield per plant (YPP; gm) and 1000 seed weight (TSW; gm) at the time of harvesting. Standard crop management practices were followed during field evaluation.

Statistical analysis

Chi-square method was used as per Singh and Chaudhary (1977) to test the goodness of fit for segregating ratios observed for leaf hairiness and flower colour. The distribution of traits and simple statistical parameters like mean, range and standard deviations were calculated as per standard statistical methods. The heritability in broad-sense (h^2) was computed as per Mahmud and Kramer (1951). The formula of Burton and DeVane (1953) was used to calculate genotypic coefficients of variation (GCV) and phenotypic coefficients variation (PCV) and categorized as per classification of Sivasubramanian and Menon (1973). Environmental variance was obtained from parental genotypes and was subtracted from F_2 variance to obtain genotypic variance Burton and DeVane (1953) and Johnson et al. (1955).

Results and discussion

Segregation pattern of flower colour and leaf hairiness

Analysis in both the crosses revealed that the characters segregated in a ratio of 3:1 (violet: white flower; hairy: smooth leaf (Table 1). In F_2 segregating population of Smooth 25 × HG 2-20, a total of 143 plants were detected as hairy while 51 plants were smooth (P= 0.70) and 146 plants produced violet flower while 48 plants had white flower (P=0.99). Similarly, in F_2 population of the cross Smooth 22 × HG2-20, 76 plants were detected as hairy and 23 plants smooth (P= 0.68) and that of flower colour, 74 plants produced violet flower while 25 plants had white flowers (P=0.95). Segregation analysis indicated monogenic inheritance for both traits in both the populations with hairiness and violet flower colour being dominant. The cosegregation ratios for flower colour and leaf hairiness obtained in the population from Smooth 25 × HG2-20 (hairy violet 105: hairy white 38: smooth violet 41: smooth white 10) and Smooth 22 × HG2-20 (hairy violet 60: hairy white 16: smooth violet 15: smooth white 8) followed the Mendelian segregating ratio expected for independent assortment (9:3:3:1) with a high chi-square probability.

Monogenic inheritance of hairiness (Sohoo and Gill 1976; Memon 1980; Chaudhary and Singh 1980) and flower colour (Memon, 1980) was reported in earlier studies also. The independent inheritance of the two traits has been reported for the first time in the present study. Role of one gene in *Brassica rapa* (Rahman 2014) and three genes in oats (Nava et al. 2010) have been implicated for the hairy trait. A number of QTLs/ genes controlling leaf hairiness have also been detected through molecular analysis in *Arabidopsis* (Payne et al. 2000), wheat (Dobrovolskaya et al. 2007) and soybean (Du et al. 2009). Inheritance studies on variations in hair density and length that were apparent in guar genotypes also might implicate more genes/ QTLs reported in other crops.

Table1. Inheritance pattern of pubescence and flower color in cluster bean

Trait/cross	Smooth 25 x HG2-20			Smooth 22 x HG2-20		
	Observed	Expected	Chi square	Observed	Expected	Chi square
Pubescence (3:1)						
Hairy-3	143	145.5	0.043	76	74.25	0.041
Smooth-1	51	48.5	0.129	23	24.75	0.123
χ^2 P value	**P= 0.70**		**0.172**		**P=0 .68**	**0.164**
Flower color (3:1)						
Violet-3	146	145.5	0.002	74	74.25	*0.0008*
White-1	48	48.5	0.005	25	24.75	*0.0025*
χ^2 P value	**P= 0.99**		**0.007**		**P=0.95**	**0.0034**
Co-segregation (9:3:3:1)						
Hairy violet-9	105	109.13	0.156	60	56	0.2857
Hairy white-3	38	36.38	0.073	16	18.5	0.2162
Smooth violet-3	41	36.38	0.588	15	18.5	0.4864
Smooth white-1	10	12.13	0.372	8	6	0.6667
χ^2 P value	**P= 0.70**		**1.189**		**P=0.646**	**1.655**

Performance of the population and genetic components of traits

The mean performance and the descriptive statistics of traits studied in the F_2 population with both parental genotypes are given in Table 2. Except for pods per

Table 2. Descriptive statistics of phenotypic values observed in F_2 population derived from the cross Smooth 25 x HG-2-20 and their parental lines

Trait	Smooth 25 (P1) Mean	HG 2-20 (P2) Mean	F_2		P_1 vs. P_2 Pr> F	P_1 vs. F_2 Pr> F	P_2 vs. F_2 Pr> F
			Mean	Range			
CMS	7.33±1.36	6.66±1.36	7.95±1.84	3-14	*	*	**
CB	15.5±2.58	16.5±2.42	12.50±9.04	1-48	*	**	**
BPP	6.5±1.76	7.5±1.37	3.55±2.95	0-11	*	**	**
PPC	6.5±1.04	6.16±0.98	5.22±1.60	2-10	ns	**	**
PPP	84.83±27.77	92.5±25.01	67.28±44.41	13-334	ns	*	**
PL	6.23±0.50	5.67±0.39	5.68±0.57	4.47-7.76	**	**	ns
TSW (gm)	34.33±1.50	33.33±1.21	32.86±3.66	15.61-41.98	*	**	*
SPP	9±0.94	8.2±0.5	8.19±0.97	5.75-9.75	*	**	ns
PH (cm)	74±12.06	80.16±5.87	91.38±14.36	58-140	*	**	**
YPP (gm)	15.09±4.75	17.19±6.48	10.04±7.05	1.72-50.34	*	**	**

CMS = Clusters on main stem; CB = No. of clusters on branches; BPP = Branches per plant; PPC = No. of pods per cluster; PPP = No. of pods per plant; PL = Pod length; TSW = 1000 seed weight; SPP = Seeds per pod; PH = Plant height; YPP = Yield per plant; *Significant at 5% level of significance; **Significant at 1% level of significance; ns = non-significant

cluster and pods per plant, both parents exhibited significant ($p < 0.05$) difference for traits under investigation. Female parent Smooth 25 was found significantly superior for test weight, clusters on the main stem, pod length and no. of seeds per pod while male parent HG 2-20 exhibited a substantially higher value for yield per plant, plant height, branches per plant and no. of clusters on branches. A substantial variation among F_2s was recorded for the characters observed in the present study. The analyses also revealed that the differences in mean of pod length and no. of seeds per pod of HG 2-20 and F_2s were non-significant. The differences in mean performance of F_2s and female parent were significant for all the traits.

The values of GCV, PCV, h^2 and genetic advance for the traits are given in Table 3. Estimation of the GCV and PCV components aids to judge the magnitude of genetic variation that exists in a population for the character *per se* (Paul et al. 2018). A high estimate of PCV compared to GCV was documented for all the traits except test weight and clusters on branches. The PCV was consistently greater than its corresponding GCV demonstrating that the obvious variability in characters may not only be because of genotypes but climate as well as soil condition were also significant in expressing the studied traits. A similar phenomenon was also reported in guar earlier by Boghara et al. (2016). Differences between PCV and GCV for test weight and clusters on branches were narrow which suggested an insignificant role of environmental factors on the expression of both traits, indicating that phenotypic selection can be done for improvement of these traits. High GCV and PCV were recorded for yield per plant, no. of branches per plant, no. of clusters per branch, no. of pods per cluster and no. of pods per plant. Kumar and Ram (2015) also recorded higher GCV and PCV for pods per cluster. Test weight and plant height showed moderate level of GCV and PCV. Low GCV and PCV were recorded for seeds per pod and pod length. Cluster on main stem had moderate level of GCV and higher PCV. Moderate to low GCV and PCV has also been reported for test weight and plant height earlier by Boghara et al. (2016).

The information on heritability supports to discern the magnitude of expression of genes under a specific environment. Moreover, the estimates of heritability along with genetic coefficients of variation would help in predicting gain under selection (Rukhsar et al. 2017). As per classification of Robinson et al. (1949), all traits exhibited moderate to high heritability. In current study, heritability ranged between 24.36% for seeds per pod to 92.32% for cluster on branches. Except, yield per plant, cluster on main stem, pod length and seeds per

pod, all traits showed high heritability. Traits with high and stable heritability can be effectively selected for any environment (Dalal et al. 2017). Characters with greater heritability are frequently governed by limited genes/QTLs with major effects. Current information on heritability suggested that direct selection for traits investigated in the present case will be more effective.

Frequency distribution and gene actions in the F_2 population

The frequency distributions for the traits studied are given in Figs. 3 and 4. The histogram of the F_2 population denotes the frequency distributions of traits value in the line. The distribution patterns advocated that characters were being managed by one major genomic part with minor/modifying genes contributing to quantitative differences. Earlier results on variability and diversity study especially with SSR markers indicated that genetic base of guar is narrow as in other legumes. Therefore, there is a need to broaden the genetic base through mutation and hybrid breeding. Though, existing released varieties show high-plasticity, yet further improvement appears to have touched plateau (Kumar et al. 2016). In the current study, significant differences were observed in various traits studied in both the parents; however, their range was narrow. Presence of transgressive segregants in F_2 population indicated the possibility of creating desired variability in different traits (Table 2 and Figs. 3 and 4). The existence of transgressive segregants is due to the accumulation of allelic recombination between both linked and unlinked alleles (Kumar and Gupta 2017). Both favorable and unfavorable alleles are dispersed between the parental lines. High value transgressive segregants are produced due to the accumulation of favourable allelic recombinations, whereas, the low value transgressive segregants are produced due to the unmasking of recessive deleterious alleles due to inbreeding (Singh et al. 2018; Brigs and Allard 1953; Rick and smith 1953). Eventually, for all characters, distribution pattern and transgressive segregations indicated the polygenic inheritance of traits studied during present investigation.

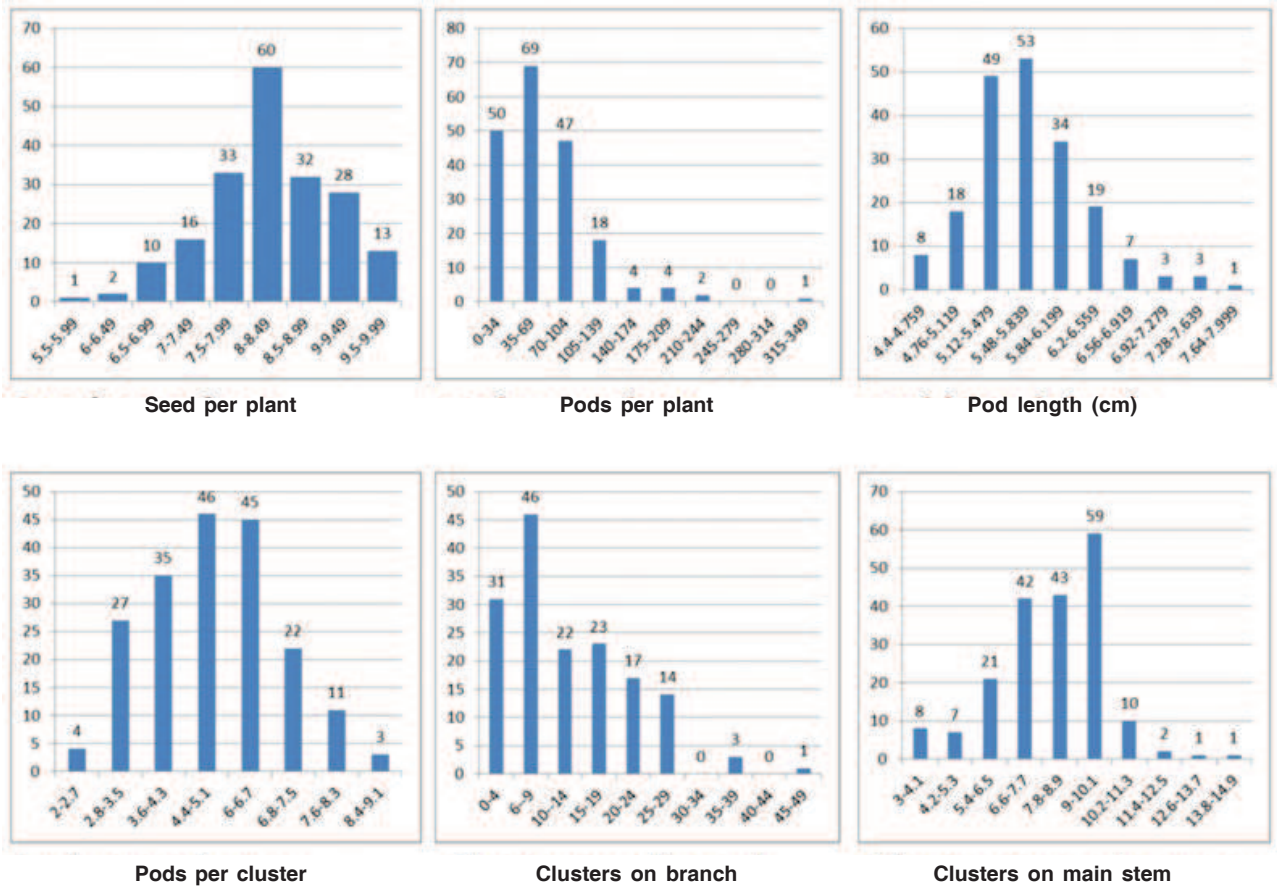

Fig. 3. Frequency distribution of no. of seed per plant, no. of pods per plant, pod length, pods per cluster, clusters on branch and cluster on main stem in F_2s of Smooth 25 × HG-2-20 based mapping population

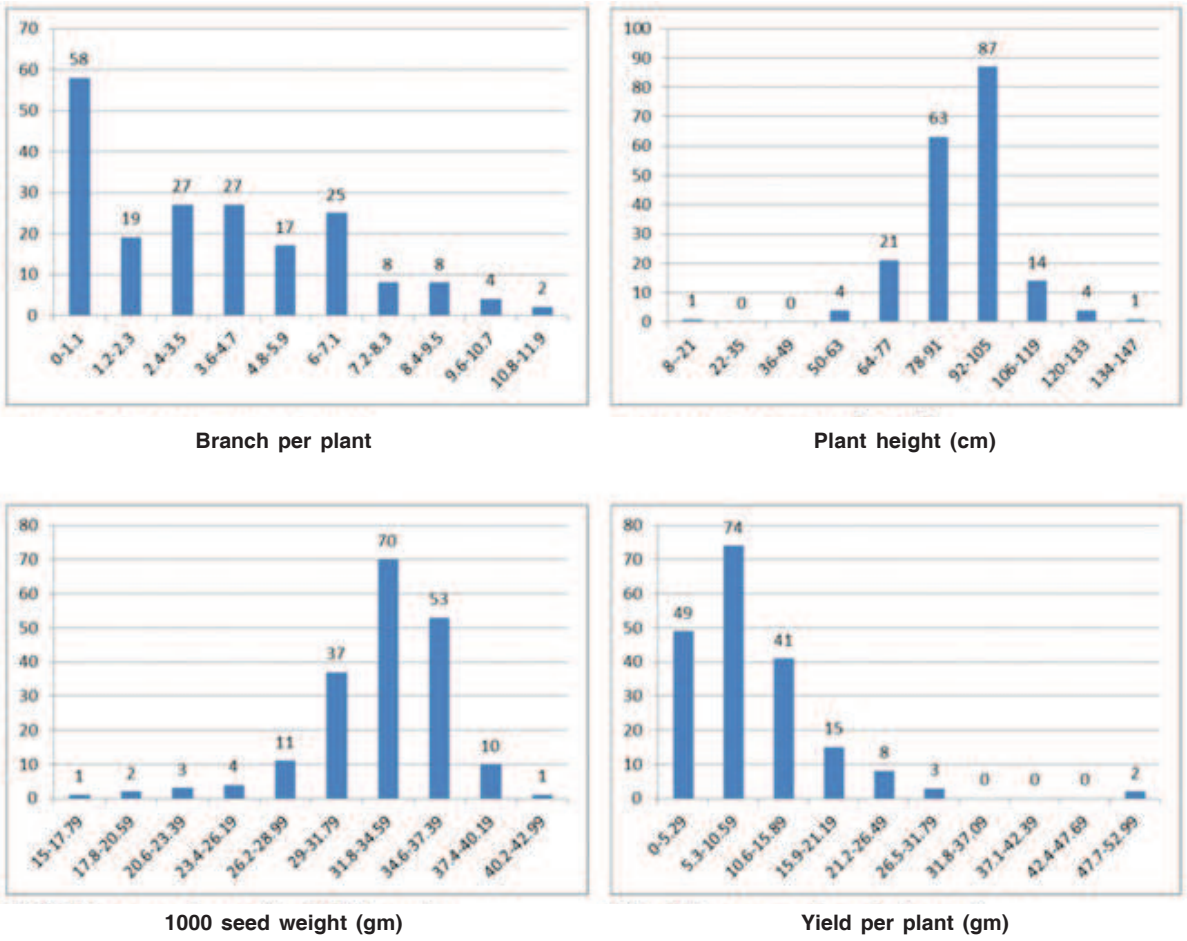

Fig. 4. Frequency distribution of branches per plant, plant height, 1000 seed weight and yield per plant in F_2s of Smooth 25 × HG-2-20 based mapping population

Table 3. Genetic components and gene action for various agronomic traits in guar

Trait	Genetic components				Skewness	Gene action as per skewness	Kurtosis	Inheritance as per kurtosis
	Heritability (%)	GCV	PCV	GA				
CMS	44.96	15.53	23.15	1.71	-0.03	Duplicate	3.48	Oligogenic
CB	92.32	69.49	72.32	17.21	0.94	complementary	3.70	Oligogenic
BPP	70.87	68.37	81.22	4.21	0.54	complementary	2.43	Polygenic
PPC	59.95	23.8	30.74	1.98	0.33	complementary	2.88	Polygenic
PPP	64.78	53.13	66.01	59.27	2.07	complementary	10.11	Oligogenic
PL	37.82	6.18	10.05	0.45	0.71	complementary	3.96	Oligogenic
SW	86.45	10.38	11.16	6.53	-1.33	Duplicate	3.97	Oligogenic
SPP	24.36	4.77	9.66	0.40	-0.27	Duplicate	3.01	Polygenic
PH	65.62	12.73	15.72	19.42	-0.94	Duplicate	5.70	Oligogenic
YPP	43.71	46.44	70.25	6.35	2.34	complementary	9.36	Oligogenic

CMS = Clusters on main stem; CB = Clusters on branches; BPP = Branches per plant; PPC = No. of pods per cluster; PPP = No. of pods per plant; PL = Pod length; TSW = 1000 seed weight; SPP = No. of seeds per pod; PH = Plant height; YPP = Yield per plant; GCV= Genotypic coefficients of variation; PCV = Phenotypic coefficients of variation

The gene action for the characters in the F_2 population was deduced through third (skewness) and fourth-order statistics (kurtosis) (Table 3). The coincidence of asymmetry in the histogram through skewness in the plot and transgressive segregation suggested the epistatic interactions (Kumar et al. 2018). Sign of skewness gives a clue about the gene action for a trait. Except for test weight, plant height, cluster on the main stem and seed per pod had a positive skewness for remaining traits suggesting the existence of complementary epistatic gene action. This also suggested that for traits with positive skewness genetic gain will be more if intensive selection will be deployed and vice-versa (Snape and Riggs, 1975). On the other hand, genetic gain will be faster with mild selection for test weight, plant height, cluster on main stem and seed per pod as these traits exhibited negative skewness. The genetic loci governing the trait with skewed pattern tend to be primarily dominant irrespective of whether they have increasing or decreasing effects on the expression of trait. Traits with leptokurtic (K = >3) histogram are typically control by a small number of segregating genomic loci and characters displaying value to K = <3 (platykurtic) commonly symbolize traits that are organized by several genes.

The study determined the genetic control of pubescence flower colour. High GCV, the appearance of transgressive segregants and involvement of fewer major genes in defining the quantitative traits including yield provided an opportunity for their genetic improvement. However, the paucity of diversity in guar suggests a need for the creation of new diversity through mutagenesis and wide hybridization.

Authors' contribution

Conceptualization of research (RS, HRM); Designing of the experiments (HRM, RS, KK); Contribution of experimental materials (RS, HRM); Execution of field/ lab experiments and data collection (HRM); Analysis of data and interpretation (SK, HRM, RS); Preparation of manuscript (RS, SK, KK).

Declaration

The authors declare no conflict of interest.

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