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Variability and genetic diversity among selfed lines (S_1) of onion (*Allium cepa* L.)

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Onion is a highly cross-pollinated crop, high variability could possibly come from traditional seed production by out-crossing or by open pollination and it could lead to more diversity and variation. The high phenotypic, genotypic variation coefficients reveal high-quantifiable variation of traits in S_1 lines. Selection of lowest premature bolting and split bulbs producing genotypes, least incidence of purple blotch incidence and thrips incidence with the highest weight of ten bulbs and maximum plot yielding genotypes were more appropriate for genetic improvement of onion. The traits aided the yield witnessed high traits heritability (h^2) and maximum genetic-advance-mean (GAM) and isolation of S_1 lines in terms of selection indices fixed for higher values of measurement. Whereas traits like premature bolting, split bulbs, purple blotch incidence, thrips incidence and were selected at least values although these traits were high heritability (h^2) and GAM could contribute for additive-gene-action and hence it indicates the straight mass selection be more effective for genetic improvement of onion genotypes or lines. The genetic distance was highly flanked by Cluster-II and –IV groups and was highly divergent. Hence, the selection of parental lines from these groups is more appropriate for traditional heterosis breeding.

Keywords: Genetic diversity, Onion, S_1 lines, Variability

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Onion botanically known as *Allium cepa* L. of the *Alliaceae* family is a major crop of *Allium* genus. The daily dietary demand of onion is increasing due to the nutritional[1], medicinal and therapeutic uses like anti-platelet aggregation, sugar reduction, availability of soluble fiber fructans, flavonoids, quercetins, and γ-*glutamyl-transpeptidase,* and also due to its cardio-vascular and anti-cancer health benefits[2]. These attributes vary among the onion population, hence there is a need to sort similar or most likely individuals for better human palatability and their improvement through traditional breeding.

Traditionally, the estimates of heritability (h^2) aid in plant selection, thus it is anticipated to exploit the genetic-variability among the population[3]. The low, high value of coefficient-of-variation (CV) and genetic-variance (GV) of traits are the cause for selection breeding[4]. Thus heritability (h^2) is not sole parameter in selection breeding, since it also depends on high with low GV. Nevertheless, high genetic gain is noted only with high genetic-variance[5]. High broad-sense h^2 indicates that high phenotypic-variance (PV) may aid for genotypic-variance (GV) and the trait differences of genotypes are real, with less environmental influence, thus selection is most effective based on phenotypic traits. High h^2 noted for height of onion plant, neck-thickness and equatorial-diameter of bulbs. However, moderate h^2 was noted for the days-to-maturity and dry-matter content[4]. The h^2 for dry-matter (52.60%) highly correlated with soluble-solids (36.10%). The breeding S_1 lines aid to the expression of genetic-variation (GV), thus identification of genotypes superior in agronomic performance is in need[5].

Genetic diversity is esteemed widely in crop improvement however estimation of diversity is always a difficult assignment. Genetic diversity estimation is utmost important in a variable population to know how closely they are linked together and it computes divergence of differentials at intra- and inter-cluster levels. Multivariate analyses lead to the estimation of diversity, it can employ where the traits overlap, where conventional methods are ineffective[6-7]. Ecological-diversity has been suggested being the index of genetic diversity[8,9]. Thus, the cultivars of different localities could be included for hybridization, and is most important to the plant breeders to recover segregates[10],

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whereas, Rameshbabu and Patil[11] find no direct relationship in geographical-distribution with genetic-diversity among crops of diverse breeding systems. Genetic-diversity facilitates diversified goals of plant breeding to escalate the yield, attractive quality broad adaptation with pests and diseases resistance. The genetic assortment of parents in breeding has huge importance for recombination breeding and is vital for crop improvement.

Material and Methods

The trial was conducted in *kharif*-2018 at Vegetable Crops Research field, College of Horticulture, Bengaluru, UHS campus, GKVK, Karnataka, India. Experiment was laid according to the Randomized Block Design (RBD) in 2 replications of 30 selfed (S_1) lines of onion, and check variety Bhima super. The nursery was raised in a poly house in May 2018. Experiment was laid-out in June 2018 with gross plots of size of length 1.5 m×1.2 m width. Plants were planted 15 cm apart of rows and 10 cm among plants for bulb crop. The crop management package of practice was followed as procedure laid down by UHS, Bagalkot[12]. Observations were recorded and subjected for statistical analysis-of-variance, by using OPSTAT open-access software, and results were interpreted with significance at five per cent[13]. The GCV, PCV interpreted as low (0-10%), moderate (11-20%) and high (>20%), heritability (h^2) and GAM sorted as low (0-30%), moderate (31-60%) and high (>60%)[14,15]. The genetic-divergence estimated based on D^2 statistic[16] using the data of S_1 lines. Mahalanobis (D^2) distance between the populations was normalized, normalized date was used for clustering through Ward's minimum variance technique, the intra-, and inter-cluster distances were calculated with SAS V.9.3 software.

Isolation of genomic-DNA

The genomic-DNA was isolated from young leaves of S_1 lines[17] with insignificant modifications. The DNA concentrations estimated using Nano-Drop-2000 (Thermo-Scientific, USA), DNA quality was assessed using agarose-gel-electrophoresis (0.8%). Further, the DNA was diluted to the concentration of 20 ng/µL for PCR assay.

SSR analysis

The 30 S_1 lines were validated using three simple sequence repeats, PCR assay performed in Bio-Rad I-Cycler machine at 10 µL volume reaction mixture with template DNA 20 ng. The PCR lined up by 94°C for four minutes of denaturation, 94°C for one minute as thirty-five successive cycles, 72°C annealing for one minute and 72°C for five minutes final extension. The amplicons were visualized on 3% agarose-gel and amplicon size was calculated using 1 kb Plus O'GeneRuler DNA ladder[18].

Results and Discussion

Genotypic and phenotypic coefficient-of-variation

The significant variance among the population possibly leads to exploitation of high breeding potential[19]. In the study, the variance of traits were varied from low to high among the selfed (S_1) lines of onion and varied from 2.42 to 43.35% for GCV respectively and 3.41 to 46.10% for PCV respectively during *kharif* 2018 presented in Table 1. Furthermore, to confirm phenotypic variability, selfed lines of onion were subjected to DNA fingerprinting with three highly polymorphic SSR markers[18]. ACM008, ACM154, ACM078 has three, four and three alleles, respectively. The number of alleles for different individuals showed variability with particular SSR markers (Fig. 1). DNA fingerprinting among the S_1 lines revealed the existence of genetic variability, the polymorphic variation among the population revealed the variation potency of the selfed lines, and linked markers with phenotypes would be utilized in marker assisted breeding for future onion improvement. The high GCV, PCV leads to subsistence variation of traits in S_1 lines. Selection of lowest premature bolting and split bulbs producing genotypes, least incidence of purple blotch incidence and thrips incidence with the highest weight of ten bulbs, and maximum plot yielding genotypes were more appropriate for genetic improvement of onion. The traits with moderate GCV were witnessed in leaf diameter, polar-, and equatorial diameters, reducing and total sugars and plot yield. Traits which witnessed moderate PCV are height of plant, leaves, leaf length diameter, neck-girth, polar- and equatorial diameter, pyruvic acid, reducing sugar, total sugars and plot yield. The moderate GCV, PCV reveal prevalence of reasonable quantity of traits variation and it could help breeder to isolate desirable variable characters in the existing variability. The GCV, PCV estimates closer, parallel showed least influence of environment, though PCV is more than GCV. Hence, it relies upon phenotypic variability for improvement. The higher GCV, PCV variation showed the persistence of genetic, and phenotypic variations for growth, quality and yield factors. Thus, broad variation of traits paves better selection efficiency and improvement of genotypes[20-26].

Heritability (h^2) and Genetic Advance Mean (GAM %)

Genetic-advance in selection breeding is based on genotypic mean values of the families over foundation population. Hence, components of heritability (h^2) and genetic-advance were important tools for breeders to plan for plant ideotypes concepts. The heritability (h^2) differed from 24.72 to 97.52% and genetic advance mean (GAM) ranged from 2.94 to 83.57% among selfed lines of onion genotypes during *kharif* 2018 (Table 1). The yield and yielding traits witnessed high

Table 1 —Genetic variability of traits among selfed (S_1) lines of onions

Characters	GCV (%)	PCV (%)	Heritability (h^2)	GAM (%)
Plant height (cm)	8.16^{L}	10.96^{M}	55.47^{M}	12.52^{M}
Number of leaves	8.26^{L}	13.25^{M}	38.82^{M}	10.60^{M}
Leaf length (cm)	8.66^{L}	11.74^{M}	54.37^{M}	13.15^{M}
Leaf diameter (mm)	13.66^{M}	17.99^{M}	57.67^{M}	21.37^{H}
Stem girth (mm)	8.74^{L}	13.88^{M}	39.67^{M}	11.35^{M}
Polar diameter (cm)	13.04^{M}	13.23^{M}	97.09^{H}	26.47^{H}
Equatorial diameter (cm)	11.84^{M}	12.06^{M}	96.37^{H}	23.94^{H}
Neck fall (50%)	6.40^{L}	6.48^{L}	97.52^{H}	13.02^{M}
Neck fall (75%)	7.13^{L}	7.25^{L}	96.78^{H}	14.45^{M}
Premature bolting (%)	43.35^{H}	46.10^{H}	88.41^{H}	83.97^{H}
Split bulbs (%)	38.42^{H}	42.27^{H}	82.59^{H}	71.92^{H}
Total soluble solids (^{0}b)	2.87^{L}	5.78^{L}	24.72^{L}	2.94^{L}
Pyruvic acid (µ mol/g)	15.49^{M}	20.95^{H}	54.66^{M}	23.59^{H}
Reducing sugar (%)	11.74^{M}	12.56^{M}	87.35^{H}	22.60^{H}
Total sugars (%)	2.42^{L}	3.41^{L}	50.12^{M}	3.52^{L}
Non-reducing sugars (%)	13.54^{M}	15.08^{M}	80.58^{H}	25.04^{H}
Purple blotch incidence (%)	38.96^{H}	40.32^{H}	93.38^{H}	77.56^{H}
Thrips incidence (%)	25.29^{H}	26.61^{H}	90.30^{H}	49.50^{H}
Weight of ten bulbs (kg)	19.02^{M}	21.38^{H}	79.13^{H}	34.86^{H}
Yield (t/ha)	13.41^{M}	14.54^{M}	85.15^{H}	25.50^{H}

L-Low, M-Medium, H-High

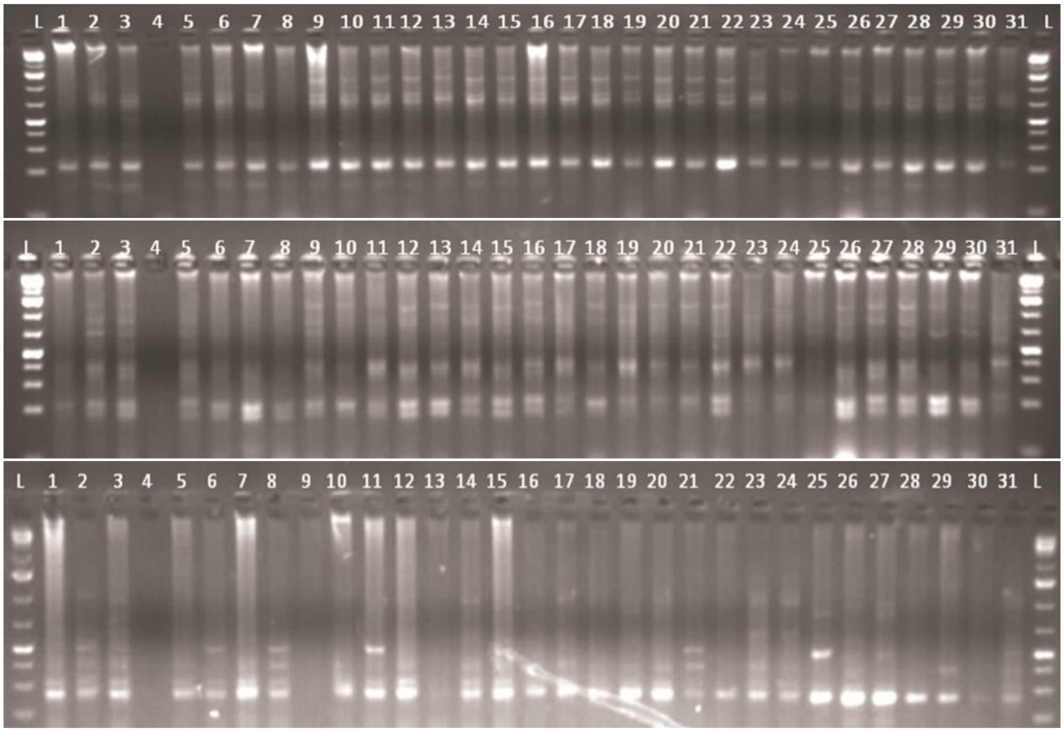

Fig. 1(a-c) — Markers amplification revealing the existence of variability among the selfed lines of onion
a – SSR Marker ACM008; b – SSR Marker ACM154; c – SSR Marker ACM078

Table 2 — Intra- and inter-cluster D^2 values of selfed (S_1) lines of onions

Cluster	I	II	III	IV
I	7.33	8.95	8.59	9.19
II		8.28	9.94	11.55
III			6.39	7.22
IV				6.38

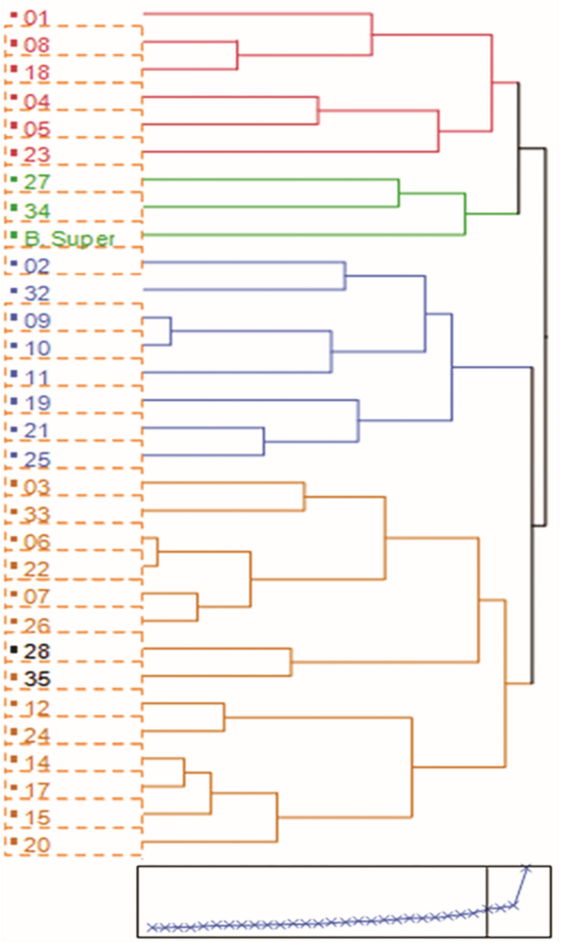

Fig. 2 — Diversity dendrogram of S_1 lines during *kharif* 2018 by Ward's minimum variance method

h^2 with high GAM (Table 1), the selection of indices selfed lines based on yield traits need to be fixed for higher values. Whereas the traits like premature bolting, split bulbs, purple blotch incidence and thrips incidence were indexed for least values. Although these traits were high heritability, GAM could contribute for additive-gene-action indicates selection is most effective in improvement of onion genotypes or lines. The traits which registered moderate effects of heritability (h^2) and genetic-advance-mean (GAM) were height of onion plant, leaves, leaf length and diameter. The traits acknowledged moderate h^2 with a low GAM to neck girth and non-reducing sugars. Thus low heritability (h^2) with low GAM was noted by total soluble solids. Traits with hypothetically non-additive-gene-action indicate that the selection might have a negligible influence on onion improvement. High h^2 with high genetic advance interpret the additive-gene-action, thus simple mass selection is appropriate to advance those traits[4,22]. Hosamani *et al.*[25] reported for average bulb weight and the bulb yield was observed by Balareddy[27], Gurjar and Singhania[4], Ananthan and Balakrishnamoorthy[24] and Hosamani *et al.*[25]. The h^2 estimates specify the selection effectiveness to exploit genetic variability[3]. Heritability alone is not a measure; since heritability (h^2) can be even high with as low as genetic-variance (GV). Nevertheless, high genetic gains were noted only with high genetic-variance[5]. High broad-sense h^2 reveal the utmost phenotypic variance caused to genotypic variance, where differences of traits in genotypes were least affected by environment, thus simple mass selection of phenotypes can be most effective. The high h^2 and GAM are important genetic factors under selection. However, characters showing high heritability do not need to have high genetic advance, since yielding traits are highly complex and inter-dependent on other traits.

Genetic divergence among selfed lines

Genetic divergence implied by magnitude of D^2 values vary from 6.38 to 11.55 in *kharif*-2018 for selfed lines of onion. Thirty selfed lines with a variety were grouped into four clusters with intra- and inter-cluster D^2 values (Table 2). The diversity analysis of S_1 lines are grouped into four-clusters (Fig. 2), the highest inter-cluster (11.55) distance noted among Cluster-II, -IV. The cluster diversity reveals that the genotypes are not restricted to a particular geographical location, although the majority of genotypes are collected from Northern Karnataka. These genotypes showed a wider genetic base among the clusters probably caused due to the free gene flow through cross-pollination in open-pollinated genotypes. This revealed the genotypes are highly divergent; this could be due to the maximum contribution of characters towards divergence. Genetic diversity necessarily assembles the varied plant breeding goals such as high yield with attractive quality, wider adaptation with diseases, and pests' resistance. Thus, the selection of S_1 lines from the cluster-II and -IV were appropriate for hybridization for better exploitation of heterosis. The genetic assortment of parental lines in plant breeding has utmost value due to recombination of traits[21,28,29]. The maximum diversity noted among S_1 lines, shown their

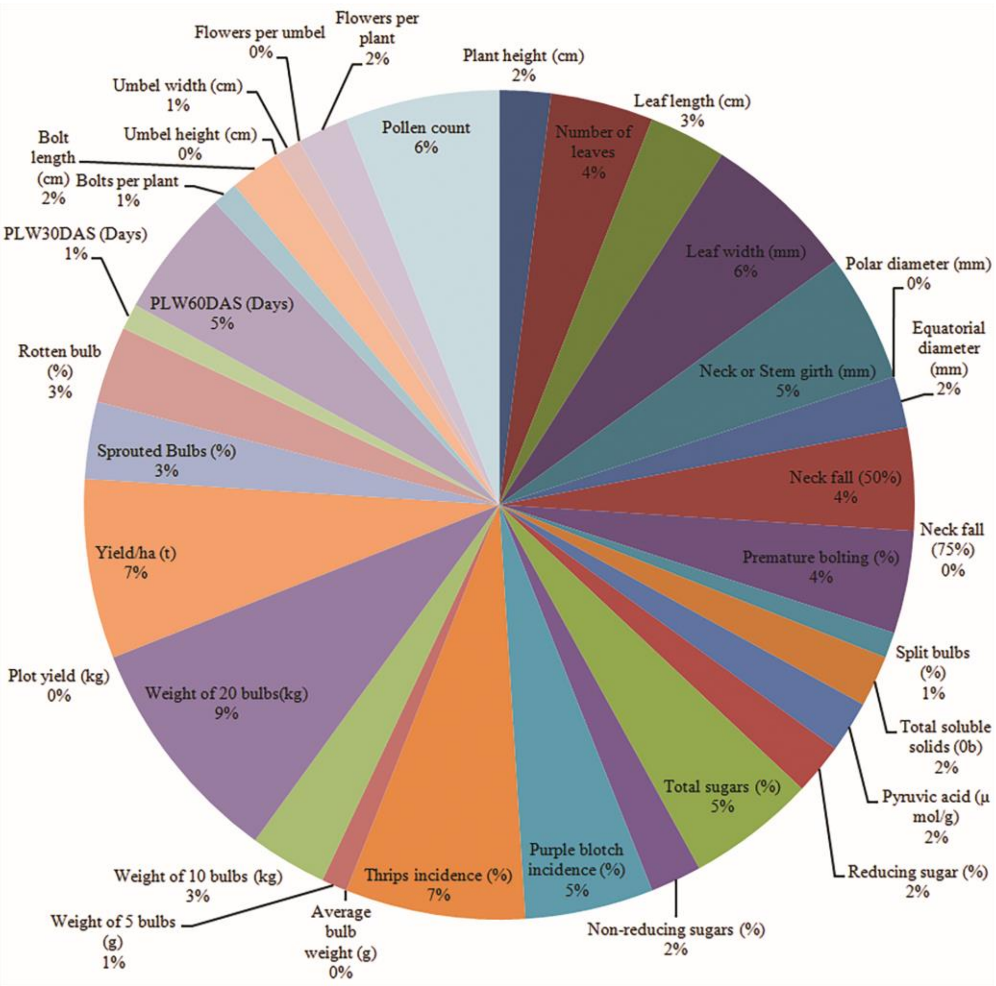

Fig. 3 — Contribution of various traits to genetic diversity in selfed lines of onion genotypes during *kharif* 2018

worth to improve qualitative, and quantitative traits in onions; accordingly, parents could be selected for hybridization[30].

Contribution of thirty-five characters in terms of percentage towards divergence of selfed lines of onion was presented in Figure 3. The cluster means for different traits in the study had revealed the substantial genetic differences between the groups. Based on the results obtained, the genotypes from the Cluster-II were superior in traits of plant growth that is plant height, leaf length and diameter, neck, or stem-girth with better bulb yielding traits like polar and equatorial diameter, weight of bulbs and plot yield. Genotypes of Cluster-II are least responsive to premature bolting and split bulbs. These genotypes are least infected by thrips incidence and purple blotch disease incidence. Furthermore, these showed the lowest physiological weight at 30 and 60 days after storage with least number of sprouted bulbs. Genotypes from Cluster are highly responsive for flowering traits in turn could lead to higher seed yield. However, the genotypes are grouped in Cluster-II and Cluster-IV with high inter-cluster distance. High yielding genotypes are grouped in Cluster-II, this could be probably due to the fact that diverse genotypes showed vigorous growth and development with least incidence of purple blotch disease and thrips. Hence in view of this, the genotypes of Cluster-II *i.e.*, COHBONC27, COHB-ONC34, Bhima Super were suited to be used in the further breeding programme for genetic improvement of onion yields. These findings were lined with researchers, alike the highest contribution for divergence from bulb weight was reported by Mohanty[28]. While, Patil[21] registered weight of ten bulbs, marketable yield and sprouting traits contributed maximum onions diversity. Ningadalli[28] witnessed bulb weight and yield, leaves, and storage loss were highest contributed traits to genetic diversity in onion. The fresh bulb weight, equatorial-diameter, split bulbs, yield of bulb, and loss in bulb weight were the highest contributing traits for divergence in onion[31,32].

These population will be tool for heterosis breeding programme for hybrid development and these diverse population also indicated the conservation of gene pool over period of time by traditional seed production method.

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Conflict of Interests

Authors declares no conflict of interest with this manuscript

Author Contributions

MDC & AM: Conceptualization, Experimentation, Formal analysis, Original draft; VR: Statistical Analysis & Interpretation of data; KJ, KA, SKS & LHB: Review & Editing of Manuscript.

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