

ASSESSMENT OF GENETIC VARIABILITY FOR YIELD ENHANCING PARAMETERS IN GINGER (*ZINGIBER OFFICINALE*) GENOTYPES.

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DOI: <https://doi.org/10.5281/zenodo.22027094>

Keywords

Article History

Received: 16 May 2026

Accepted: 12 June 2026

Published: 20 August 2026

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Abstract

Ginger (*Zingiber officinale* Rosc.) being a member of Zingiberaceae family is cultivated as a herb and spice crop. The present research experiment, comprising of 8 ginger genotypes of diverse origin, was conducted in semi controlled tunnel conditions under RCBD with three replications in research area of Vegetable Research Institute, AARI, Faisalabad, Pakistan. The data was recorded for plant height, leaf length, leaf width, number of leaves per plant, number of tillers per plant, tiller thickness, rhizome weight per plant, rhizome length, rhizome width, rhizome thickness, inter nodal distance of leaves and total yield per hectare. The analysis of variance (ANOVA) showed highly significant variability for all the traits and portrayed VGC-20008 as a best ginger genotype to be utilized for further ginger improvement programme on account of high mean values for number of leaves per plant, leaf length, rhizome weight per plant, rhizome thickness, inter nodal distance of leaves and total yield per hectare. Most of the traits exhibited highly significant (at 1%) correlation with total yield per hectare on both genotypic and phenotypic level. Path coefficient analysis revealed positive and direct effects on rhizome yield per hectare for various plant attributes. The magnitude of phenotypic coefficient of variance was higher than the genotypic coefficient of variance for all the plant characters and maximum phenotypic variance (16332.9) was found in rhizome weight per plant. All the traits exhibited more than 90% broad sense heritability and number of leaves per plant showed higher value of 99.90% heritability. Genetic advance estimation revealed highest genetic advance (144%) for total yield per hectare which exhibited additive type gene action for its expression. A principal component Bi-plot explained 85% variability through PC-1 and PC-2 and expounded VGC-20008 a superior ginger genotype for its maximum variability towards different traits such as rhizome width, number of leaves per plant, rhizome length, rhizome weight per plant and rhizome thickness. The molecular analysis through utilization of 13 SSR markers also

expressed the genetic diversity among ginger genotypes. The dendrogram presented highest similarity coefficient (0.88) for VGB-17000 and VGC-20008 indicating closed genetic relationship among these genotypes. Thus the morphological and molecular assessment of genetic variability among ginger genotypes would be further utilized in future for genetic improvement of ginger germplasm and evolution of high yielding varieties.

INTRODUCTION

Ginger (*Zingiber officinale* Rosc.) is a perennial herbaceous and monocotyledonous plant commonly grown as annual. Traditionally, ginger has been used for thousands of years as a medicine and a spice crop plant by ancient Asians (Purselove *et al.*, 1981). It was initially originated in the region of Indo-Malayan and then dispersed to other regions of world (Purselove *et al.*, 1981). It was one of the first spices to have been exported from Asia, arriving in Europe with the spice trade, and was used by ancient Greeks and Romans. From India, it was also carried by traders into the Middle East around the 1st century CE (Common Era). It was primarily grown in southern India, along with peppers, cloves, and numerous other spices. Currently, countries i.e., India, China, Malaysia, Thailand, Australia, Brazil, Bangladesh, Cameroon, Costa Rica, Fiji, Ghana, Guatemala, Hawaii, Jamaica, Nepal, Mauritius, New Zealand, Philippines, Nigeria, Sierra Leone, Sri Lanka, Taiwan, Trinidad and Uganda are producing ginger in large quantity at commercial level (Kizhakkayil and Sasikumar, 2011).

Ginger is as member of *Zingiberaceae* family which has erect stem and well-developed long smooth leave sheaths. Stem botanically known as rhizome which is an edible portion and it is soft and tender in early stages of use when immature. But when matures or store for prolonged period of time it becomes hard, thick laterally, compressed often branched and is about 1.5-2.5 cm in diameter depending on variety and agro-climatic conditions, etc. It has linear, glabrous and sessile leaves, yellowish green flowers, cylindrical spikes, oblong capsule shaped fruits and white to yellowish brown rhizomes. The adventitious roots arise from the nodes of the scale present on the rhizome on the top layer of the soil. Whereas,

true roots are long and have a large number of root hairs which absorbs water and minerals from the soil. The flowers are produced in some countries where the agro-ecological conditions are favorable but seldom seen in Malaysia (Jaidka *et al.*, 2018).

Ginger is an important herbal and highly nutritious plant, widely use as medicine. A 100g of ginger rhizome powder contains saponin (4.01g) and cyanogenic glycoside (2.81mg) and low amount of phytin (0.28g), tannin (0.28mg), oxalate (0.26g), crude fiber (10.36%), ash (6.57%), ether extract (6.48%) and crude protein (5.45%). Mineral elements like manganese, zinc, calcium, copper, sodium, iron, phosphorus and potassium are also found in its powder (Ogbuewu *et al.*, 2014). Traditionally ginger was used to cure the diseases of gastrointestinal tract and as appetizer. Ginger spice is an anti-inflammatory, antioxidant plant which regulates urine system, and controls high blood pressure. Moreover, the consumption of ginger is effective for nausea and vomiting during pregnancy (Ali and Gilani, 2007).

Ginger is the plant of tropical and sub-tropical climate, which grows well in warm and humid climate up to an altitude of 1500m from sea level. For the successful cultivation of crop, ginger requires well distributed rainfall throughout the growing period and one month of dry weather essential for harvesting of the crop. Ginger requires organic enriched sandy loam to clayey soil with proper aeration and good drainage capacity (Ravindran *et al.*, 2002).

Ginger is completely sterile plant and breeding of ginger is still impractical. Chromosome aberrations like chromosome inversions and translocations are responsible for sterility. It is much difficult to obtain seeds from ginger plant. Although, different techniques like, bud

pollination, removal of stigmatic surface or by the application of pollen germination medium to stigmas etc., were applied by many scientists around the globe yet no viable seed of ginger could be obtained. Tetraploid ginger plants have higher in vitro pollen germination percentages as compared to the diploid plant but seed settings on the tetraploids ginger have not been reported yet (Shinichi Adaniya, 2001). Thus, well developed ginger rhizomes are used as seed material (Ravindran *et al.*, 2007).

Ginger commonly propagated through vegetative rhizomes and develops adventitious or lateral roots emerged from its nodes (Hayden *et al.*, 2004). The size and weight of ginger rhizome also varies for different genotypes grown under different locations as large rhizomes produce high yield in ginger (Hailemichael and Tesfaye, 2008; Kandianan *et al.*, 2010; Whiley, 1990). In Asia, growers use 15-75 g ginger rhizomes to raise the crop (Ravindran and Babu, 2005). Whereas, ginger can also be grown through pieces having two to four well developed buds (Rafie and Mullins, 2018).

The ginger germination takes about 50 days from sowing (Ravindran and Babu, 2005) and dormant rhizomes take more time to germinate and result in poor sprouting (Sanewski, 1996). Soaking of ginger rhizomes in tap water at ambient temperature for 24 hours, ten days prior to the planting is recommended as good treatment to increase sprouting (Ravindran and Babu, 2005).

Rhizomes of ginger are usually stored in the well ventilated containers at 12 -14 °C with 60%-70% relative humidity. It is pertinent to mention that ginger storing temperature should not be lower than the 12 °C as ginger rhizomes are chilling sensitive (Paull and Cheng, 2015). Ginger rhizomes can be stored for 90 to 105 days in the ventilated polythene bags (Ravindran and Babu, 2005). Storability of ginger rhizomes is limited and if its storage for prolong period of time would generate the issues of decreased starch content and weight loss (Chung and Moon, 2011) and even at 10 % weight loss the shriveling of ginger rhizomes occurs that deteriorates its

quality (Paull *et al.*, 1988). Seed rhizomes are stored placed in sand or silt medium to obtain uniform germination for 30-45 days. Furthermore, ginger rhizomes are treated with hot water (50°C) to control fungal attack and to ensure the optimum germination (Nair, 2013). After the storage ginger pieces usually planted in moist sawdust in plastic seedling trays at 25°C (Labrooy and Abdullah, 2016), and temperature above 30 °C reportedly leads to weak sprouts (Ravindran and Babu, 2005).

Globally, ginger is cultivated on an area of 392182 hectares with total production of 2785 thousand tonnes and average yield of 7.47 t/ha. India is the leading producer of ginger, producing 893 thousand tonnes from area of 172 thousand hectares with average yield of 5.192 t/ha. China is the second largest country with total production of 533 thousand tonnes from area of 479 thousand hectares with average yield of 11.129 t/ha. Pakistan is producing only 130 tonnes of ginger from area of 43 hectares with average yield of 0.46 t/ha (FAO, 2018).

Per capita consumption of ginger has increased from about 0.5 kg in 1996 to 1.7 kg in 2015 (Nguyen *et al.*, 2019). The import value of ginger in the Pakistan in 2017 increased from \$63.32 million to \$82.53 million in 2018 (FAO, 2017 & 2018). Therefore, there is a lot of potential for increasing national production of ginger in the Pakistan to escalate export graph of the country and to lower down the import bill on national exchequer.

The unavailability of graded seed, narrow genetic base of ginger germplasm, higher pH of soils and harsh climatic conditions characterized by very hot and humid summers and cool dry winters as well as minimum precipitation rate curtail the commercial production of ginger in Pakistan.

In the past, although limited research on ginger crop and inappropriate field conditions of the country hampered the commercial cultivation of ginger in Pakistan yet with the advent of tunnel technology and various irrigation systems like drip irrigation and sprinkler irrigation system etc., the cultivation of ginger may become practical at commercial scale.

In order to address these constraints of ginger cultivation and to make ginger production economical in Pakistan the present research has been conducted under semi-controlled tunnel conditions.

Material and Methods

The current research was conducted at the farm of Vegetable Research Institute, Ayub Agricultural Research Institute Faisalabad. Genotypes were planted during the year of 2020-21. The experimental material was consisted of eight genotypes of ginger which were evaluated for the genetic variability for different yield relating traits among these eight genotypes four were from Chinese origin, one Indian, one Thailand, one Burma and one Tissue cultured genotype of china source. The seed rhizomes of ginger were cut into small pieces having 2-3 buds and placed in shade for 2-3 days for the hardening of cut portions. These seed rhizomes were treated with the solution of copper oxychloride 10 gram per liter of water, Di-thene M-45 10 gram per liter of water, streptomycin Sulphate 1 gram per liter of water, for 15 minutes and put them in shade for 24 hours for drying. The treated seed rhizomes were planted in sand and after 30 days of planting these germinated seed rhizomes were transferred in the semi controlled tunnel conditions. Land was prepared by 3-4 ploughings followed by 1-2 plankings. The solarization process of land was also accomplished for the period of one month to evacuate the harmful insect pests residing in the soil. N, P and K fertilizers were applied at the rate (370.5:123.5:370.5) kg per acre respectively. The total fertilizer requirement was fulfilled using Ammonium Sulphate, Single Super Phosphate, Phosphoric Acid and Sulphate of Potash. The basal dose of Ammonium Sulphate, Single Super Phosphate and Sulphate of Potash was applied at the time of sowing. Rest of the fertilizer requirement was fulfilled via drip irrigation system on weekly bases throughout the growth period of crop. The Genotypes were planted under semi controlled tunnel conditions covered with green net having 50 percent of shade capacity. The experimental material was sown

under RCBD with three replications. Humidity was raised to 65% using fogging system installed in the tunnel. A Plot size of 9m² (12m × 0.75m) was maintained for each replication. The plant into plant distance was 20 cm whereas row to row distance was kept of 75 cm. Mulching is an important practice to conserve the soil water and keep the soil temperature under suitable range required for good ginger cultivation. In the present study rice straw was used as mulch after sowing of ginger genotypes. Irrigation was done through drip irrigation system to keep the soil in wet conditions. In addition to mulching other cultural practices i.e. earthening up and hoeing etc. was accomplished throughout the cropping period. The growth of ginger starts to retard when temperature drops below to 15°C. The leaves of plants start withering and drop after drying. Genotypes of experiment were harvested during the month of December after drying of leaves. The data was recorded for plant height, leaf length, leaf width, number of leaves per plant, number of tillers per plant, tiller thickness, rhizome weight per plant, rhizome length, rhizome width, rhizome thickness, inter nodal distance of leaves and total yield per hectare. The recorded data of all traits was analyzed using Statistix version 8.1 (Steel *et al.*, 1997). While correlation of all traits with rhizome yield, path analysis, variance components, broad sense heritability, genetic advance and Principal component bi-plot analysis was computed through R Studio version 1.4.1717.0 (Popat *et al.*, 2020).

Molecular analysis

For the molecular analysis, leaf samples were taken from the guarded plants and the samples were stored at -80 °C temperature. SSR markers were selected on the bases of their previous performance for genetic diversity (Table 1) (Das *et al.*, 2017). Cetyl Tri-methyl Ammonium Bromide (CTAB) based DNA extraction protocol was used to isolate the DNA prior to molecular analysis Allen *et al.* (2006). DNA quantity was measured by using the nano drop method (ND 2000, Thermo Scientific, U.S.A.). PCR reaction

was conducted in thermal cycler with total reaction volume of 25 μ L including 20 ng/ μ L genomic DNA of each variety, 0.6 μ M of each forward and reverse primers and 12 μ L of green master mix for different SSR markers. The following temperature conditions were applied for amplification: initial denaturation 94°C for 5 min, 35 cycles of denaturation 94°C for 1 min, annealing at variable temperatures according to primers for 1 min (Table 2), extension at 72°C for 2 min. Final extension at 72°C for 10 min. The amplified products were stored at 4°C.

Amplified products were separated by 6% agarose gel electrophoresis and photographs were obtained using the Bio-Imaging System (Biorad, USA).

The data for SSR markers were recorded in the form of binary matrix. The presence of band was scored as 1, whereas the absence was scored as 0. Polymorphism was detected among eight genotypes of ginger on the basis of alleles with NTSYSpc 2.2 version and dendrogram was generated. The genetic diversity levels of eight ginger genotypes were assessed at the end.

Table 1: primers used for Molecular analysis

Primer name	Forward primer	Reverse primer	Annealing temp. °C
SSR-CBT02	TCCTCCCTCCCTTCGCCCCACTG	CGATGTTGCGCCATGGCTGCTCC	55
SSR-CBT03	ATCAGCAGCCATGGCAGCGACC	AGGGGATCATGTGCCGAAGGCC	55
SSR-CBT04	ACCCTCTCCGCCTCGCCTCCTC	CTCCTCCTCCTGCGACCGCTCC	60
SSR-CBT05	CTCTGTCTCCTCCCCCGCGTCG	TCAGCTTCTGGCCGGCCTCCTC	60
SSR-CBT06	GCCTCGAGCATCATCATCAG	ATCAACCTGCACTTGCCTGG	55
SSR-CBT07	CGATCCATTCTGCTGCTCGCG	CGCCCCCATGCATGAGAAGACG	55
SSR-CBT08	TTCCCTGTAAAGAGAGAAATC	GTGTATTTGGTGAAAGCAAC	60
SSR-CBT09	CTCTGTCTCCTCCCCCGCGTCG	TCAGCTTCTGGCCGGCCTCCTC	60
SSR-ZOF22	TAAGTGGCCCTGGAACAAAC	GGTGCAGTAAACCAGGATGAA	55
SSR-ZOF53	CAGCGTCACTATCTCCACCA	ACGGGGATCTTAGGCTTGTT	50
SSR-ZOF161	AAGGCCCTCGACATCTGTAA	TACGGGAGGAGTGACCACA	55
SSR-ZOF183	ATCGAATCCATCCCCTGAA	CAGGTCGGAGGTGAAGTCTC	50
SSR-ZOF302	TTCGACACCACCAGAACAGA	GTCCATGGCGTACACCTTCT	55

Table 2: PCR profile for primer optimization

Steps	Temperature	Time	Cycles
Initial denaturation	94°C	5 min	1

Denaturation	94°C	1 min	35
Annealing	50-60 °C	1 min	
Extension	72°C	2 min	
Final Extension	72°C	10 min	1
Hold	4°C	∞	Until turned off

Results and Discussion

All the parameters showed highly significant results for plant height, number of leaves per plant, number of leaves per tiller, leaf length, leaf width, tiller thickness, number of tillers per plant, inter-nodal distance, rhizome length, rhizome width, rhizome weight per plant, rhizome thickness and total yield expressed highly significant variability among all genotypes. These results were similar to the findings of Basak *et al.*, 2019; Saikia and Shadeque, 1992; Anargha *et al.*, 2021. The results of ANOVA concluded that all studied traits could be considered in selection criteria for yield improvement of ginger genotypes.

Mean data was computed for 12 yield related plant attributes of 8 different genotypes of ginger grown under semi controlled tunnel conditions (Table 3). On the basis of mean comparison among 8 genotypes of ginger for all the traits, it could be explained that the selection of VGC-20008 for number of leaves per plant (144.37), leaf length (20.35cm), rhizome weight per plant 411gm), rhizome thickness (32.83mm), inter nodal distance of leaves (2.77cm) and total yield per hectare (9.14 T/ha) would be effective to ensure improvement in ginger germplasm whereas utilization of VGC-19005 would be effective for the improvement of tiller thickness, number of tillers per plant and rhizome length.

Table 3: Mean values of eight genotypes for different yield related traits in ginger

Genotypes	PH	NL/P	LL	LW	TilT h	NT/ P	Rhwt/ P	Rhl	Rhw	RhTh	IND	TY/ h
VGI-17002	27.00 _d	26.13 ^h	15.9 _{8^b}	1.15 _e	5.35 _d	3.46 ^e	76.67 ^f	14.46 _{cd}	2.86 _e	26.71 ^c _{de}	1.48 _c	0.89 _c
VGT-18004	22.48 _e	56.53 ^e	10.8 _{8^c}	1.50 _d	4.46 ^e	7.33 ^d	104.67 _e	13.42 _d	3.33 _d	24.48 ^e	1.18 _d	0.60 _c
VGB-17000	12.31 _f	46.13 ^f	10.5 _{4^c}	1.42 _d	4.04 ^e	4.46 ^e	71.00 ^f	15.56 _{cd}	3.34 _d	25.74 ^d _e	1.15 _d	0.62 _c
VGC-20008	36.87 _{bc}	144.3 _{7^a}	20.3 _{5^a}	2.17 _{bc}	7.84 ^a _b	9.80 ^c	411.00 _a	23.9 ^a	5.56 _a	32.83 ^a _a	2.77 _a	9.14 _a
VGC-19005	35.12 _c	113.1 _{4^b}	20.2 _{8^a}	2.25 _{ab}	8.07 ^a	12.53 _a	312.67 _b	24.26 _a	3.53 _{cd}	31.12 ^a _b	2.59 _{ab}	6.68 _b
VGC-18003	39.68 _b	36.33 ^g	14.9 _{0^b}	1.98 _c	5.60 _d	10.03 _{bc}	214.67 _c	19.53 _b	3.66 _{bc}	29.03 ^b _c	2.72 _a	6.54 _b
VGC-17001	38.12 _{bc}	92.60 ^c	19.5 _{4^a}	2.42 _a	6.47 ^c	11.80 _{ab}	163.33 _d	16.33 _c	3.36 _d	28.19 ^c _d	2.57 _{ab}	6.30 _b

VGCTc- 19007	45.43 _a	68.13 ^d	15.1 _{0^b}	1.52 _d	7.84 ^b	12.13 _a	324.00 _b	20.13 _b	3.82 _b	29.03 ^b	2.32 _b	6.50 _b
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PH = Plant Height, NL/P = Number of leaves per plant, LL = Leaf length, LW = Leaf width, TilTh = Tiller Thickness, NT/P = Number of tillers per plant, Rhwt/P = Rhizome weight per plant, Rhl = Rhizome length, Rhw = Rhizome width, RhTh = Rhizome thickness, IND = Inter nodal distance of leaves. TY = Total yield per hectare

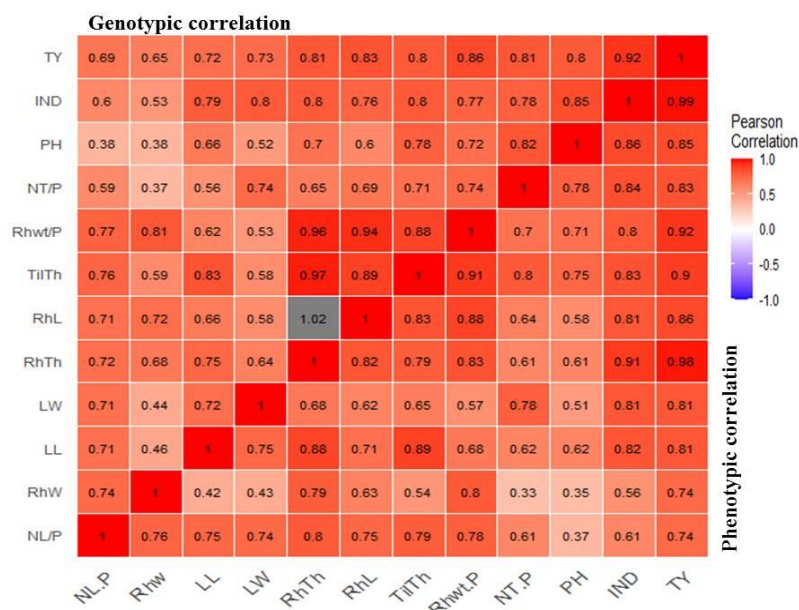
Correlation Analysis

All the plant characters exhibited highly significant (at 1%) genotypic correlation with total yield per hectare except number of leaves per plant, leaf length, leaf width and rhizome weight per plant which were also significant (at 5%) with total yield per hectare. All the plant traits under study showed highly significant (at 1%) phenotypic correlation with the total yield per hectare (Table 6).

Plant height revealed positive and highly significant correlation with total yield

(0.85**). The maximum values of correlation was observed by inter nodal distance of leaves (0.87) in relation with plant height which exhibited that more plant height with more inter nodal distance of leaves possessing more leaves at each node would result in more assimilation of photosynthesis to be accumulated in underground stem. These findings were supported by Sasikumar *et al.*, 1992; Islam *et al.*, 2008; Lincy *et al.*, 2008. Number of leaves per plant showed the positive and significant correlation with some traits and the maximum value was observed in rhizome thickness (0.81*) and tiller thickness (0.79*) which concluded that with the increase in number of leaves per plant would result in improvement of rhizome thickness and tiller thickness which are yield determining plant characters. These results were supported by Ravi *et al.*, (2017).

Table 6: Genotypic and Phenotypic correlation for growth, yield and its component characters in ginger genotypes



PH= Plant Height, NL/P= Number of leaves per plant, LL=Leaf length, LW= Leaf width, TilTh= Tiller Thickness, NT/P= Number of tillers per

plant, Rhwt/P=Rhizome weight per plant, Rhl=Rhizome length, Rhw=Rhizome width,

RhTh=Rhizome thickness, IND= Inter nodal distance of leaves

Path coefficient analysis:

Path coefficient analysis of 8 diverse ginger genotypes revealed that all traits had positive direct effects on rhizome yield per hectare at both genotypic as well as at phenotypic level except leaf width, tiller thickness and rhizome weight per plant which showed negative direct effects on total yield per hectare at genotypic level while rhizome weight per plant showed negative direct effects at phenotypic level only on total yield per hectare (Table 7). Plant height showed the maximum positive direct effect (0.45926) on yield at genotypic level. Thus selection on the bases of plant height could be effective for yield improvement. Number of leaves per plants, Leaf length, Leaf width, tiller thickness, number of tillers per plant, rhizome weight per plant, Rhizome length, Rhizome width, Rhizome thickness, inter nodal distance of leaves expressed positive indirect effects on yield at both levels. This proved that these parameters will be considered fruitful in yield enhancement through indirect selection. Some of the results were found relevant with the findings of Islam *et al.*, (2008).

Genetic variance and phenotypic variance

The maximum value of phenotypic variance was determined in rhizome weight per plant (16332.9) followed by number of leaves per plant (1664.04) and plant height (115.08). All these findings indicated that phenotypic variance includes both genotypic and environmental variance thus selection on the bases of phenotypic variance would be effected by different environment under which these selections were made for improvement of ginger genotypes (Table 8).

Genotypic and phenotypic coefficient of variation

Similarly, the magnitude of phenotypic coefficient of variance was higher than the genotypic coefficient of variance for all the plant characters. The maximum value of phenotypic coefficient of variance was observed in total yield

(72.89) followed by the rhizome weight per plant (60.93) and number of leaves per plant (55.94). The similar trend was observed in genotypic coefficient of variance as maximum genotypic coefficient of variance was expressed by total yield (71.28) followed by rhizome weight per plant (60.82) and number of leaves per plant 955.91). All the findings indicated that additive type of gene action was involved in the expression of these characters and high magnitude of PCV exhibited that selection of these traits phenotypically would be effective for the genetic improvement of rhizome yield (Table 8).

Broad sense heritability

All the traits under study exhibited more than 90% broad sense heritability. Maximum magnitude of broad sense heritability was expressed by number of leaves per plant (99.90%) followed by rhizome weight per plant (99.63%) and inter nodal distance of leaves (98.32%). These findings concluded that selection based on these characters would be significantly useful to improve the rhizome yield of ginger genotypes, while minimum value of broad sense heritability (91.07%) showed by rhizome thickness (Table 8).

Genetic advance

The estimation of genetic advance revealed that total yield showed maximum value of genetic advance (144%) followed by rhizome weight per plant (125%) and number of leaves per plant (115%). These findings concluded that additive type gene action was involved in the expression of higher GA possessing plant attributes. The rhizome weight per plant was expressed high value (99.63%) broad sense heritability along with high GA (115%) which indicated that phenotypic selection of ginger genotypes based on rhizome weight per plant would be effective for improvement of ginger genotypes. Furthermore, the minimum GA (18%) was observed in rhizome thickness like broad sense heritability (91.07%). These findings were supported by Pandey and Dobhal, 1993; Umar *et al.*, 2017, (Table 8).

Principal Component Bi-plot analysis of 8 ginger genotypes

Data of 8 ginger genotypes was collected from the semi controlled tunnel conditions experiment for 12 traits and their mean values were computed. In this present analysis the first two principal components explained 85% of the total variation (Table 9). The first PC which accounted 76.21 % of the total variation, was the average effect of traits such as tiller thickness (0.311), rhizome weight per plant (0.308), rhizome thickness (0.315), inter nodal distance of leaves (0.309) and total yield (0.323). This perhaps emphasizes the significance of these traits for the overall genetic diversity in PC1. The second PC which accounted for 9.154 % of the total variation among genotypes was the average effect of traits such as number of leaves per plant (0.347) and rhizome width (0.571). These findings were supported by the findings of Momina *et al.*, (2011).

PCA-Biplot analysis was plotted for all traits and expressed that VGB-17000, VGT-18004 and VGI-17002 showed in the same group, which means that they are genetically close, VGC-17001, VGC-18003 and VGCTc-19007 were in the other same group and they were genetically close genotypes for the traits such as leaf length, leaf width, inter nodal distance of leaves, number of tillers per plant and plant height, while VGC-20008 found to be most diverse genotype and showed the maximum variability towards different traits such as rhizome width, number of leaves per plant, rhizome length, rhizome weight per plant, rhizome thickness (Fig 1).

SSR polymorphism of Markers

A total of 13 SSR markers were used to evaluate the genetic diversity of eight genotypes of ginger. A sum total of 52 alleles with a mean value of 4.72 alleles per locus were amplified. The microsatellites were highly polymorphic showing 30 polymorphic alleles with an average of 2.72 alleles per locus. The highest number of alleles and polymorphic alleles per microsatellite were 9 for SSR CBT02 and 6 for SSR CBT04 respectively (Table 10). The results also indicated that 11 out of 13 markers (84.61%) exhibited

amplification, 10 generated polymorphic bands (90.90%) while 3 appeared to be monomorphic and 2 failed to amplify. The PIC values for 13 markers ranged from 0.44 to 0.87 with a mean value of 0.70. The most informative markers were CBT02, CBT04 and CBT03 with PIC values of 0.87, 0.86 and 0.82 respectively (Table 10).

Cluster dendrogram

The organization of genetic diversity within eight ginger genotypes was examined through cluster analysis on genomic-SSR data. Firstly similarity matrix was first generated according to SHAN similarity index and then the dendrogram was constructed.

The highest similarity coefficient was observed among the VGB-17000 and VGC-20008 (0.88), which indicated that these genotypes were genetically close genotypes and was closely regrouped. Genotypes VGC-17001 and VGCTc-19007 (0.82) which indicated that these genotypes were genetically close genotypes and was closely regrouped. VGB-17000, VGC-20008 and VGC-19005 showed similarity coefficient of (0.81) which indicated that they were genetically close genotypes. VGC-18003, VGC-17001 and VGCTc-19007 showed similarity coefficient (0.80), which indicated that these genotypes were genetically, closed genotypes. VGT-18004, VGB-17000, VGC-20008 and VGC-19005 showed similarity coefficient of (0.77) that indicated these were genetically close genotypes. VGI-17002, VGT-18004, VGB-17000, VGC-20008 and VGC-19005 were showed similarity coefficient (0.74) that indicated they were closely grouped. Genotypes VGI-17002, VGT-18004, VGB-17000, VGC-20008, VGC-19005 VGC-18003, VGC-17001 and VGCTc-19007 was showed similarity coefficient of (0.72), (Fig 2). Dendrogram constructed on the bases of genomic SSR data of all markers, showed grouping of ginger accessions to their respective area of collection and told about geographical closeness due to genetic similarity irrespective of the relationship that existing at the morphological level. These findings related to the findings of Das *et al.*, (2017).

Table 7: Genotypic path analysis for total yield and its component factors

Characters	PH	NL/P	LL	LW	TilTh	NT/P	Rhwt/P	RhL	Rhw	RhTh	IND
PH	0.459	0.109	0.249	-0.054	-0.638	0.084	0.093	0.262	0.048	-0.085	0.323
NL/P	0.175	0.287	0.283	-0.076	-0.644	0.063	0.100	0.329	0.097	-0.097	0.229
LL	0.305	0.217	0.375	-0.077	-0.724	0.063	0.088	0.312	0.059	-0.107	0.306
LW	0.242	0.214	0.234	-0.102	-0.532	0.080	0.073	0.272	0.057	-0.083	0.305
TilTh	0.361	0.227	0.335	-0.067	-0.812	0.082	0.117	0.390	0.075	-0.117	0.312
NT/P	0.380	0.177	0.234	-0.085	-0.654	0.102	0.096	0.301	0.048	-0.078	0.313
Rhwt/P	0.335	0.225	0.258	-0.058	-0.742	0.076	0.128	0.412	0.104	-0.116	0.300
RhL	0.277	0.216	0.269	-0.064	-0.726	0.071	0.121	0.436	0.091	-0.123	0.302
Rhw	0.175	0.219	0.175	-0.045	-0.479	0.038	0.104	0.314	0.127	-0.095	0.210
RhTh	0.326	0.231	0.334	-0.070	-0.792	0.067	0.123	0.446	0.100	-0.120	0.342
IND	0.399	0.176	0.309	-0.083	-0.681	0.086	0.103	0.354	0.072	-0.110	0.372

Diagonal indicates direct effects Residual=0.0539 plant, Rhwt/P=Rhizome weight per plant, PH= Plant Height, NL/P= Number of leaves per plant, LL=Leaf length, LW= Leaf width, TilTh= Tiller Thickness, NT/P= Number of tillers per distance of leaves, RhL=Rhizome length, Rhw=Rhizome width, RhTh=Rhizome thickness, IND= Inter nodal distance of leaves

Table 8: Estimates of variance components and heritability in broad sense and genetic advance as percent mean

Character s	GMS	EMS	r	Mean	α_g	α_P	GCV	PCV	Hbs	GA (at 5%)	GAM %
PH	348.60	3.37	3	32.12	115.08	116.20	33.3	33.5	99.0	21.99	68
NL/P	4992.1	4.81	3	72.92	1662.4	1664.0	55.9	55.9	99.9	83.95	115
LL	46.22	2.23	3	15.95	14.66	15.41	24.0	24.6	95.1	7.69	48
LW	0.64	0.018	3	1.80	0.21	0.22	25.3	25.7	97.1	0.92	51
TilTh	6.94	0.15	3	6.14	2.26	2.31	24.4	24.7	97.7	3.06	50
NT/P	36.75	1.22	3	8.94	11.84	12.25	38.4	39.1	96.6	6.97	78
Rhwt/P	48998.	183.0	3	209.7	16271.	16332.	60.8	60.9	99.6	262.2	125

RhL	52.04	2.02	3	18.45	16.67	17.35	22.1	22.5	96.1		
Rhw	1.97	0.023	3	3.68	0.65	0.66	21.8	21.9	98.7	8.24	45
RhTh	22.80	2.03	3	28.39	6.92	7.60	9.27	9.71	91.0	5.17	18
IND	1.48	0.02	3	2.09	0.49	0.50	33.2	33.5	98.3	1.42	68
TY	34.63	1.512	3	4.66	11.04	11.54	71.2	72.8	95.6	6.69	144
		2					8	9	3		

GMS = Genotypic mean square, EMS=Error mean square, r =Replication, α_g =Genotypic variance, α_P =Phenotypic variance, GCV = Genotypic coefficient of variation, PCV =Phenotypic coefficient of variation, Hbs =Broad

sense heritability, GA =Genetic advance, GAM%= Genetic advance as percentage of the mean

Table 9: Eigen values, total variance, cumulative variance and Eigen vectors for 12 parameters of 8 ginger genotypes

Characters	PC1	PC2
PH	0.261	-0.442
NL/P	0.272	0.347
LL	0.281	-0.093
LW	0.261	-0.185
TilTh	0.311	-0.024
NT/P	0.273	-0.382
Rhwt/P	0.308	0.198
RhL	0.299	0.190
Rhw	0.236	0.571
RhTh	0.315	0.168
IND	0.309	-0.246
TY	0.323	-0.069
Eigenvalue	9.146	1.098
Total variance %	76.216	9.154
Cumulative variance %	76.216	85.370

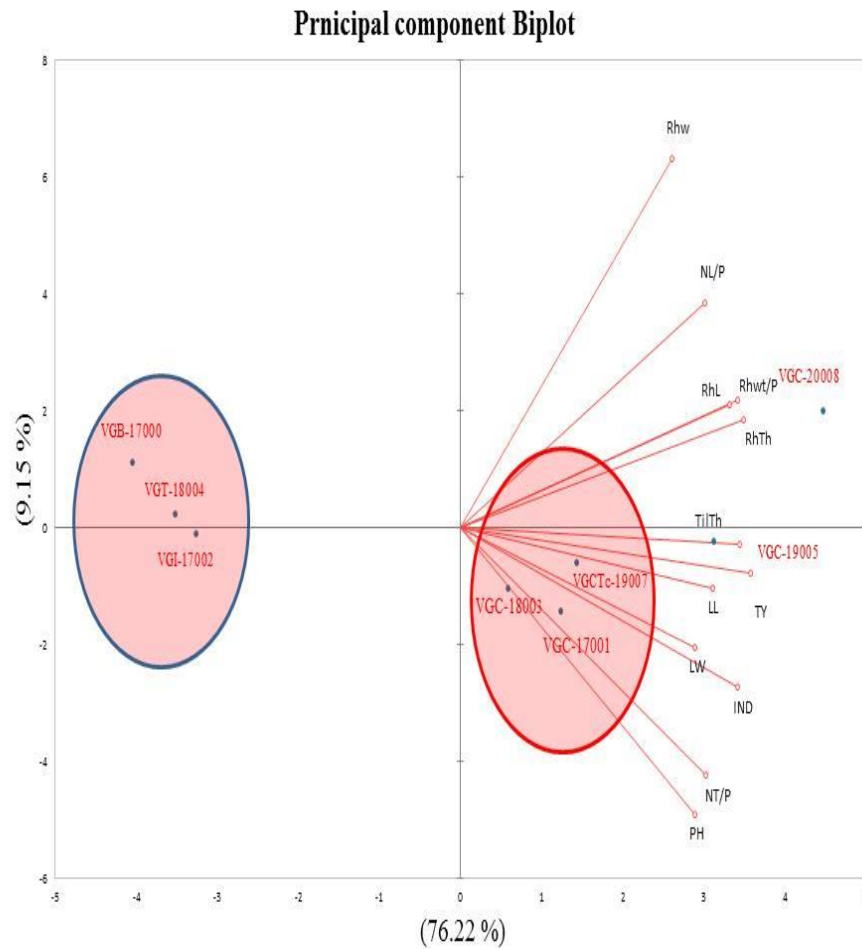


Fig 1: PCA Bi-plot of 8 ginger genotypes

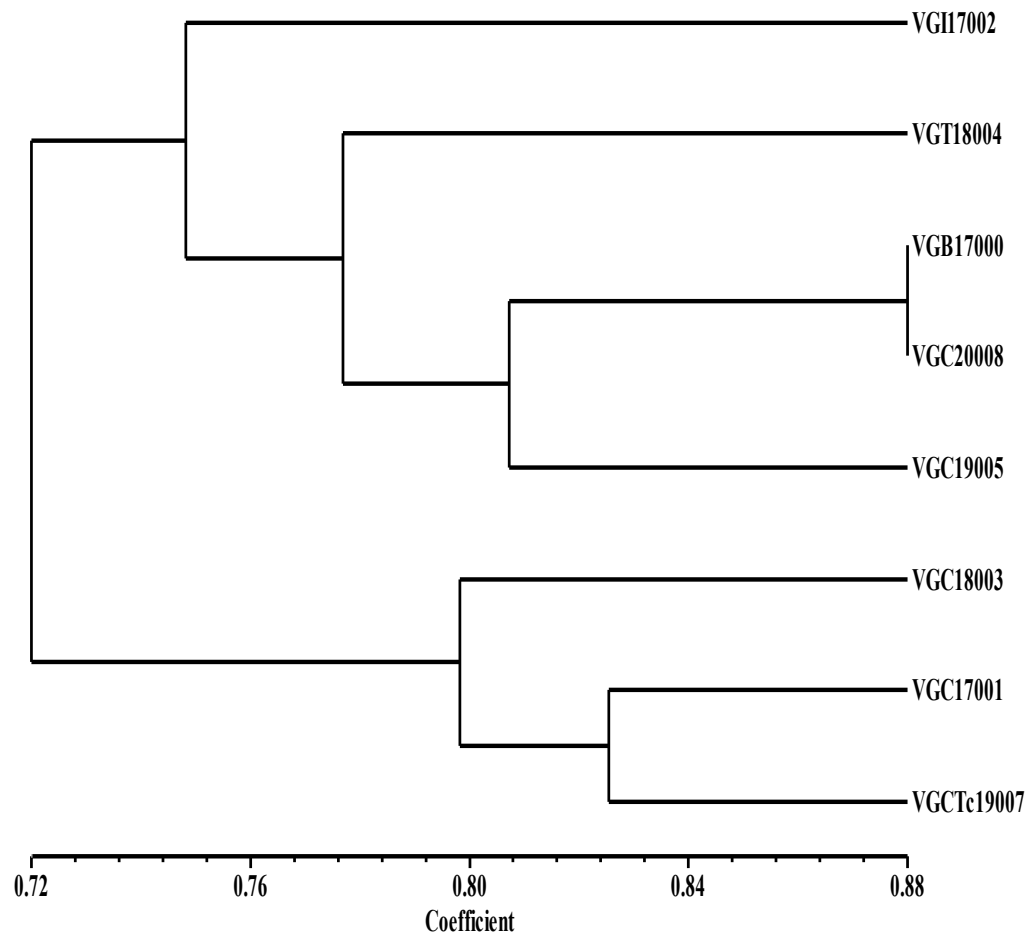


Fig 2: Cluster analysis of 8 ginger genotypes

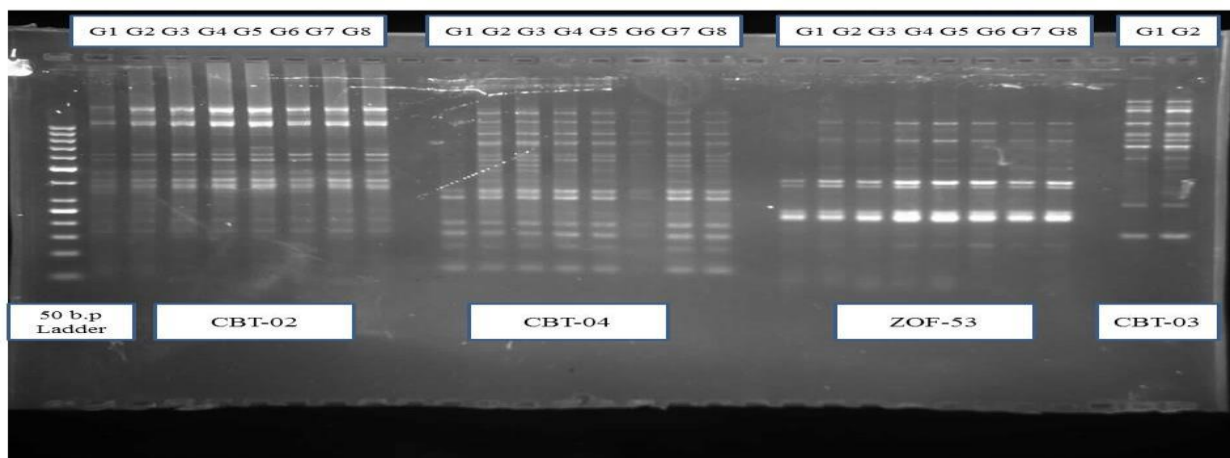


Fig 3: Segregation pattern of SSR markers (CBT-02, CBT-04, ZOF-53, CBT-03) across 8 ginger genotypes run on agarose gel with 50bp ladder

G1=VGI-17002, G2=VGT-18004, G3= VGB-17000, G4= VGC-20008, G5= VGC-19005, G6=

VGC-18003, G7=VGC-17001, G8=VGCTc-19007

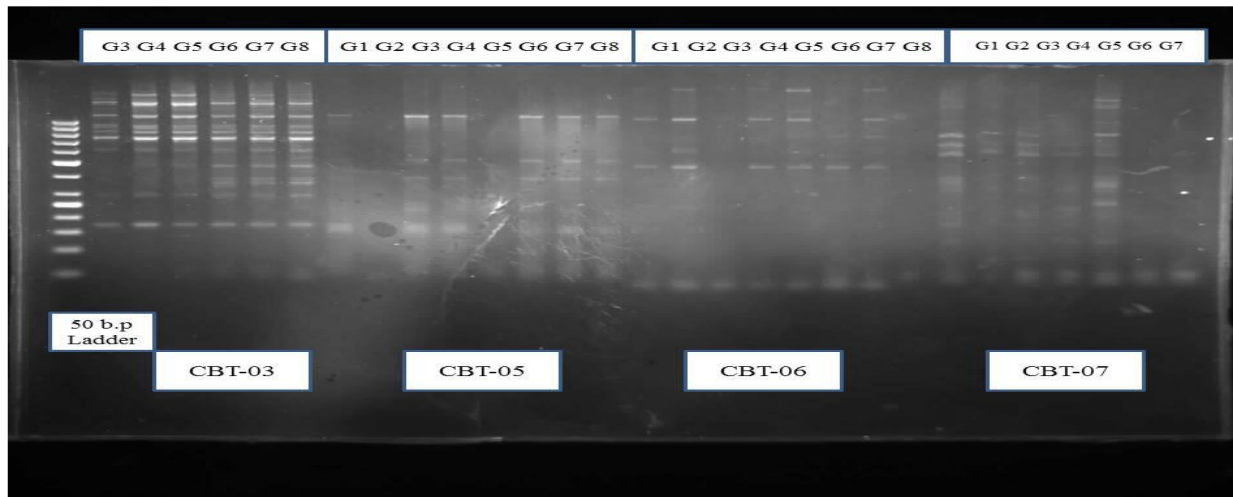


Fig 4: Segregation pattern of SSR markers (CBT-03, CBT-05, CBT-06, CBT-07) across 8 ginger genotypes run on agarose gel with 50bp ladder

G1=VGI-17002, G2=VGT-18004, G3= VGB-17000, G4= VGC-20008, G5= VGC-19005, G6= VGC-18003, G7=VGC-17001, G8=VGCTc-1900

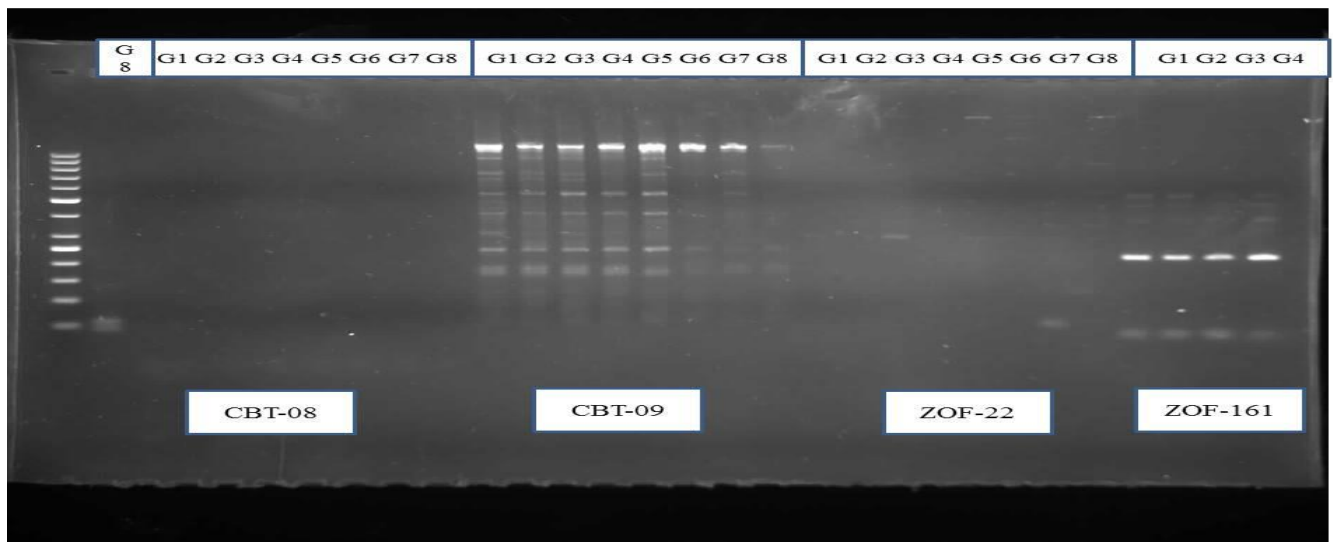


Fig 5: Segregation pattern of SSR markers (CBT-08, CBT-09, ZOF-22, ZOF-161) across 8 ginger genotypes run on agarose gel with 50bp ladder

G1=VGI-17002, G2=VGT-18004, G3= VGB-17000, G4= VGC-20008, G5= VGC-19005, G6= VGC-18003, G7=VGC-17001, G8=VGCTc-1900

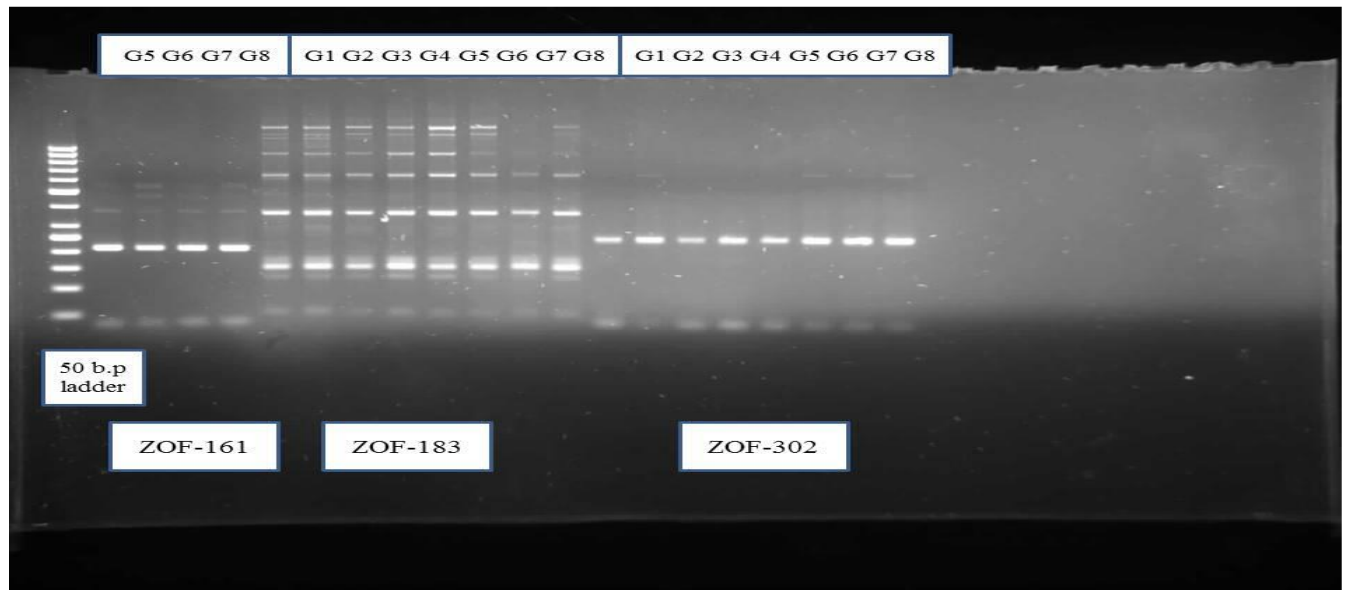


Fig 6: Segregation pattern of SSR markers (ZOF-161, ZOF-183, ZOF-302) across 8 ginger genotypes run on agarose gel with 50bp ladder

G1=VGL-17002, G2=VGT-18004, G3= VGB- VGC-18003, G7=VGC-17001, G8=VGCTc-17000, G4= VGC-20008, G5= VGC-19005, G6= 19007

Table 10: List of SSR markers used in the study along with polymorphic information content (PIC), number of alleles (NOA) polymorphic alleles (PA) and annealing temperature (Ta)

Sr. No	Marker name	PIC	PA	NOA	Ta
1.	CBT02	0.87	4	9	55
2.	CBT03	0.82	2	6	55
3.	CBT04	0.86	6	8	60
4.	CBT05	0.50	2	2	60
5.	CBT06	0.54	3	3	55
6.	CBT07	0.69	4	4	55
7.	CBT09	0.80	5	6	60
8.	ZOF53	0.79	1	5	50
9.	ZOF161	0.66	2	3	55
10.	ZOF183	0.75	0	4	50

11.	ZOF302	0.44	1	2	55
12.	CBT08	Not amplified			
13.	ZOF22	Not amplified			

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