

A report on

**CHARACTERIZATION AND EVALUATION OF
STRAWBERRY (*Fragaria x anannasa*Duch.) GERMPLASM
FOR GROWTH, YIELD AND QUALITY TRAITS
UNDER POLYTUNNEL AND SHADENET**



Submitted by: Anmol (11713764)
M.Sc. Ag. (Horticulture) Fruit Science

Supervised by:
Dr. Shailesh Kumar Singh

**SCHOOL OF AGRICULTURE
LOVELY PROFESSIONAL UNIVERSITY
PUNJAB**

CERTIFICATE

This is to certify that synopsis titled “**Characterization and evaluation of strawberry (*Fragaria x anannasa*Duch.) germplasm for growth, yield and quality traits under polytunnel and shade net**” submitted in partial fulfillment of the requirement for the award of degree of **Master of Science in Agriculture in the discipline of Horticulture (Fruit Science)**, is a research work carried out by **Anmol (Registration No. 11713764)** under my supervision and that no part of this synopsis has been submitted for any other degree or diploma.

(Signature of Student)

Anmol

Registration No.: 11713764

(Signature of Supervisor)

Dr. Shailesh Kumar Singh

UID-19105

School of Agriculture,
Lovely Professional University,
Phagwara, Punjab.

(Signature of co-advisor)

Dr. Chandra Mohan Mehta

UID-18376

School of Agriculture,
Lovely Professional University,
Phagwara, Punjab.

(Signature of co-advisor)

Dr. Vikas Kumar

UID-18677

School of Agriculture,
Lovely Professional University,
Phagwara, Punjab.

(Signature of co-advisor)

Dr. Sanjay Singh

UID- 22085

School of Agriculture,
Lovely Professional University,
Phagwara, Punjab.

INTRODUCTION

Strawberry botanically known as *Fragaria x ananassa* Duch. is one of the aggregate accessory fruit, widely grown all over the world due to the acceptance for its sweet flavour, aroma and nutritional values. It is a rich source of vitamin C and also consist of vitamin B1, B2, B3, B5, B6, E and K. Also, it contains minerals like calcium, iron, magnesium, manganese, potassium, phosphorus, sodium and zinc and have a moderate amount of carbohydrates. Other polyphenols present include flavonoids such anthocyanins, flavonols and phenolic acids such as hydroxybenzoic acid and cinnamic acid. Higher levels of flavanoids are possessed by strawberries. Only about 1% of total fresh weight of a strawberry comprises of achenes, but still 11% of total polyphenols in fruit are contributed by achenes, include ellagic acid (inhibit cancer), glycosides.

The cultivated strawberry is octaploid ($2n=8x=56$) is hybrid of two American octaploid species *F. chiloensis* and *F. virginiana*. It is mainly a temperate fruit crop but now it is also grown under subtropical and tropical conditions (Sharma and Sharma, 2004; Sharma *et al.*, 2006). The world's largest producer of strawberry is United States followed by Spain, Russia, Korea, Japan and Poland (FAOSTAT, 2007). In India strawberry is cultivated in Maharashtra, H.P., U.P., Uttarakhand, West Bengal, Delhi, Haryana, Punjab and Rajasthan.

Strawberry plants are perennial, stoloniferous herbs, meaning that they spread via stolons or "runners". The leaves are trifoliate and arise from the "crown" (a reduced stem in the center of the plant). Leaflets are ovate or broadly oval, obtuse, dentate or coarsely serrate. The runners produce "daughter" plants at every other node.

Flowers are white, about 1" across, with 25-30 yellow stamens and 50-500 pistils on a raised, yellow, conical receptacle. Borne on a dichasial cyme, the center-most terminal flower opens first and is largest, producing the largest fruit. Most cultivars are self-fruitful and therefore do not need cross pollination for fruit set. However, bee activity is beneficial in transferring pollen to stigmas in an individual flower.

The strawberry is an accessory fruit, since the edible portion is non-ovarian in origin (it is largely swollen receptacle tissue). The true fruits which contain the seed of the strawberry are achenes, which are similar to tiny sunflower seeds. The achenes are the numerous, tiny, ellipsoid specks

that cover the fruit surface. Fruit mature rapidly; ripening occurs in 20 to 50 days after pollination.

Strawberries are low-growing herbaceous plants with a fibrous root system and a crown from which arise basal leaves. The leaves are compound, typically with three leaflets, sawtooth-edged, and usually hairy. The flowers, generally white, rarely reddish, are borne in small clusters on slender stalks arising, like the surface-creeping stems, from the axils of the leaves. As a plant ages, the root system becomes woody, and the “mother” crown sends out runners (e.g., stolon) that touch ground and root, thus enlarging the plant vegetative. Botanically, the strawberry fruit is considered an “accessory fruit” and is not a true berry. The flesh consists of the greatly enlarged flower receptacle and is embedded with the many true fruits, or achenes, which are popularly called seeds.

It firstly originated in France but now the cultivation is done all over the world due to its adaptability to different environment conditions. Mostly strawberries are consumed in fresh form. The processed products like pies, syrups, ice-creams, jam, fruit juice are also available. Artificial flavouring is used in products like perfumes, hand sanitizers etc.

Many strawberry cultivars have been grown around the world and new varieties appear at frequent intervals (Nielsen and Lovell, 2000). The continued introduction of strawberry cultivars to the market increases the need for reliable methods of identification and genetic diversity assessment (Degani *et al.*, 2001). In addition, verification of strawberry cultivars is essential for growers and plant breeders to protect breeders’ rights (Garcia *et al.*, 2002). Verification is especially important in a clonally propagated crop like strawberry where one original plant of an economically important cultivar can be easily used to produce a large number of plants (Gambardella *et al.*, 2001). Strawberry cultivars have been identified using morphological traits (Nielsen and Lovell, 2000) and molecular markers (Levi *et al.*, 1994; Congiuet *et al.*, 2000; Degani *et al.*, 2001; Garcia *et al.*, 2002; Shimomura and Hirashima, 2006; Govan *et al.*, 2008; Brunnings *et al.*, 2010). Molecular marker techniques for analysis of strawberries include isozymes and hybridization-based and PCR-based DNA markers and complement the use of morphological markers in germplasm characterization.

Genetic analysis is recognized as an effective method for cultivar identification or phylogenetic systematics (Harrison & Luby, 1997; Ohtsubo *et al.*, 2002; Rajapakse *et al.*, 1992). Through the use

of DNA markers, DNA polymorphism among cultivars can be detected, making genetic fingerprinting reliable, even when the sample is only one grain. In Italy, genetic analysis was used to identify strawberry plants of the patented cultivar 'Marmolada', and it was accepted as forensic evidence (Congiuet *et al.*, 2000).

Considering the significance of biodiversity and its evaluation under different agroclimatic condition the studies has been conducted with following set of objectives:

1. To evaluate the performance of various genotypes of strawberry under polytunnel and shade net.
2. To study variability and heritability of various qualitative and quantitative traits of strawberry.
3. To determine the interrelationship among yield and yield contributing characters.

REVIEW OF LITERATURE

Evaluation of performance of 15 strawberry varieties by **Shukla *et al.* (1980)** was done in the hills of Uttar Pradesh. Cultivars Local Kalimpong, Blackmore, Albritton, Banglore, Local Jeolikote, Red Coat and Jucunda were very high in total soluble solids content and also resulted in low to medium pH values. Highest pH was recorded in Tioga and Shasta. In cultivars Banglore, Albritton, Local Kalimpong, Red Coat and Robinson highest acidity was observed and least in Tioga and Swiss. Cultivars Jucunda, Red Coat, Swiss, Gem, Stelemaster, Phenomenal, Shasta and Albritton were observed with highest ascorbic acid in order and Local Kalimpong was having least value of ascorbic acid.

The performance of nine strawberry cultivars namely, Blakemore, Catskill, Elista, Fairfax, Robinson, SengaSengana, Shasta, Tioga and Torrey were studied by **Sharma *et al.* (1981)**. Tioga had the largest fruits followed by Torrey while Fairfax produced the smallest. Tioga and Torrey were better in appearance, uniformity, fruit firmness and taste among all the cultivars. There were no marked differences in total soluble solids of cultivars. Acidity differed in cultivars considerably. Tioga showed the lowest acidity followed by cultivars Torrey and Elista. TSS/acid ratio was highest in Tioga and Torrey followed by Elista and SengaSengana.

Comparative performance of ten strawberry cultivars viz. Tioga, Torrey, Blackmore, Shasta, Elista, Fairfax, Howard-17, Florida-90, Catskill and Robinson were studied by **Joolka and**

Badiyala (1983) for three years during 1979, 1980 and 1981. The results revealed that Tioga was the highest yielder with an average yield of 9653 kg/ha. Torrey had highest berry weight (5.99 g) and TSS (7.4 °Brix). Minimum TSS was observed for Robinson. Cultivar Fairfax showed maximum acidity (1.17 %). Maximum and minimum TSS/acid ratio was recorded in cultivars Torrey and Fairfax respectively.

In an evaluation study by **Dhaliwal and Singh (1983)**, it was observed that yield per plant was highest in SengaSengana (209.56 g) whereas it was minimum in cultivar DilPasand (56.59 g). The 2nd best cultivar with respect to yield (173.90 g) was Black Rose followed by Pusa Early Dwarf (136.19 g). They also found that Katrain Sweet produced the highest number of runners followed by Pusa Early Dwarf (8.67 and 7.40, respectively). The cultivars Black Rose, DilPasand, Climax, Gorella and SengaSengana failed to produce runners. On the overall basis, Pusa Early Dwarf was found to be the most promising cultivar under Ludhiana conditions.

Evaluation of ten strawberry cultivars and fifteen selections at Beltsville, Maryland was carried out by **Galletta (1984)**. The results revealed that cultivars Allstar, Earliglow and Raritan with yields of 28,000, 25,000 and 31,900 lb/acres respectively were acceptable.

Under Hisar condition, India, **Beniwalet al. (1989)** evaluated strawberry cultivars for growth, flowering and fruiting characters. They observed that plant height ranged from 9.6 cm in Fairfax to 13.0 cm in Elista. Cultivar Blackmore produced highest number of leaves (13.9), while highest number of runners per plant was obtained in cultivar Elista (4.7) followed by cultivars Tioga (4.4), Fairfax (4.1) and Torrey (3.2).

The cropping, fruit size, quality and other characteristics of 15 strawberry cultivars were compared with Elsanta and Gorella by **Lieten (1989)**. None of the newer cultivars were considered to be an improvement over Elsanta, which yielded an average of 0.575 kg/plant with very uniform and attractive fruits.

Performance of five strawberry cultivars viz. Brighton, Douglas, Fern, Toro and Pajaro were studied under plastic greenhouse and field conditions in northern Greece by **Paraskevopoulovet al. (1990)**. Under greenhouse condition, Brighton and Douglas proved to be the most productive cultivars followed by Toro and Pajaro, while Fern was least productive one. Brighton, Fern and Toro (day neutral cvs.) gave 47, 38 and 53 percent respectively of their marketable yield during the first five weeks of harvest period while Douglas and Pajaro (short day cultivars) gave 22 and

24 per cent of their marketable yield respectively. Strawberries produced either under greenhouse or field conditions were of similar quality but yield of plants under field conditions was about double that of plants growing under greenhouse conditions.

The performance of eight day neutral and seven short day strawberry varieties was evaluated on raised beds in British Columbia by **Baumann *et al.* (1993)**. Among the day neutral strawberry varieties, maximum and minimum runner count was observed in cultivars Irvine and Tribute during first year, whereas during second year maximum runners (10) were produced by Irvine and Ozark Beauty. The leaf size was found to be maximum in Selva (58 cm²) during first year while it was maximum in Fern and Irvine (48 cm²) during second year of study. The minimum leaf size was observed in Hecker during both the years. Among short day cultivars, the maximum runner count was observed in Puget Beauty (9) and Shuswap (10) during first and second year, respectively. The leaf size was observed maximum in Shuswap (63 cm²) during first year and Sequoia (67 cm²) during second year of observation.

Studies on genetic variation and inheritance of quality and yield characters for advanced strawberry breeding was studied by **Morishita (1994)**. Heritability was high for sugar/acid ratio, titratable acidity, average fruit weight, colour, seed position and glossiness, suggesting that these should be evaluated when selecting the parents to be used in a breeding programme.

The performance of eight strawberry cultivars was assessed by **Chandel and Badiyala (1996)** under sub-tropical conditions of Himachal Pradesh. Maximum plant spread was attained by Etna and highest number of runners was produced by Belrubi. Belrubi exhibited highest fruit yield (1.64 t/ha) followed by Torrey (1.52 t/ha). Belrubi also produced large fruits which contained highest total soluble solids (8.6 %), TSS/acid ratio (10.82) and total sugars (4.73 %).

Data on total fruit yield mean, individual fruit weight and earliness for each site and season was recorded by **Faedi *et al.* (1999)** for three sites Battipaglia, Metaponto and Marsala on 13 strawberry cultivars and three pre-released selections. Generally, yields were highest at Metaponto, with 1997-1998 season giving the best results. In that year, the overall mean total yield at Metaponto was 883 g/plant ranging from 587 g for Cartuno to 1072 g for Clea. An evaluation study of 11 cultivars and ten advanced selections was conducted at Cesena by Sbrighi *et al.* (1997). Highest commercial yields were obtained from Selection 89.250.1 (1074 g/plant) followed by cultivar Marmolada (913 g).

Funaro et al. (2000) compared thirteen strawberry cultivars in open field and protected tunnels. In open fields, fruit production of cultivars Camarosa, Carlsbad, Clea and Tudla was the best with around 700-800 grams/plant and in protected tunnels fruit production PA91.201 was the best (over 500 g/plant).

Asrey and Singh (2004) evaluated strawberry cultivars under semi-arid conditions of Punjab. Highest fruit weight was recorded in Fern, while the size was better in Chandler than other cultivars. The maximum total soluble solids and lower acidity were noted in Chandler.

From the varietal performance of strawberry, **Nagreet et al. (2005)** found that acidity was highest in Sea Scape (1.15 %) while lowest was in Sweet Charlie (0.76 %). The total soluble solids were highest in Florida-90 (6.50 %) followed by Sweet Charlie (5.49 %) and Chandler (5.25 %). Highest fruit weight was recorded in Fern, while the size was better in Chandler than other cultivars.

Ram and Yadav (2006) evaluated ten cultivars of strawberry (*Fragaria x ananassa* Duch.) under Lucknow conditions. The maximum number of leaves per plant (15.65) were recorded in Red Coat, whereas minimum (8.66) in Elsanta. The maximum plant height (13.97 cm) in Chandler, whereas minimum plant height (7.90 cm) in Confictura. The maximum number of runners (5.26) were observed in Brighton, whereas minimum (3.16) in Sea Scape. The maximum number of flowers (33.11) in Belrubi, while minimum (6.44) in Sea Scape. The maximum number of fruits (16.22) in Elsanta, whereas minimum (3.56) in Florida-90. The minimum days for maturity (140.0) in Elsanta, while maximum day (169.44) in Florida-90. The minimum days for flowering (97.56) in Elsanta, whereas maximum days (142.28) in Florida-90. The maximum fruit length (2.92 cm) in Belrubi, whereas minimum fruit length (1.69 cm) in Elsanta. The maximum fruit diameter (2.83 cm) and fruit weight (4.09 g) were obtained from Selva, whereas cv. Elsanta produced the minimum fruit diameter (1.22 cm) and fruit weight (1.11 g), respectively. The cultivar Belrubi gave the highest fruit yield (198.34 g/plant) and Florida-90 lowest (19.67 g/plant). On the basis of these characteristics, the cultivars Selva, Chandler, Belrubi and Brighton are rated suitable for cultivation under Lucknow conditions.

Das et al. (2007) studied the performance of 33 promising genotypes with different mulching materials under sub-humid subtropical plateau region of eastern India. Irrespective of type of mulches, the cultivar Sea Scape recorded the maximum plant height at flower initiation stage.

With respect to leaf number, the maximum number of leaves per plant at flower initiation stage was recorded in case of cv. DilPasand. At the end of fruiting stage, the maximum average plant height on different mulches was recorded in cultivars Missionary, Sea Scape and Addie. The maximum number of leaves per plant was observed in case of cv. Missionary.

Singh et al. (2008) evaluated 25 strawberry cultivars under sub-tropical condition of Meghalaya. Cultivars Camarosa and Cherigent-5 showed earliest flowering and they took only 91 and 94 days respectively after planting. The fruits harvested per plant were highest in Dana (24.9) followed by Elista (23.3) and Blakemore (22.6) but its size was maximum in Camarosa followed by Ofra and Sweet Charlie. The highest fruit yield per plant was produced by Camarosa (248.2 g) followed by Ofra but fruit weight was higher in Camarosa (17.2 g) followed by Sweet Charlie (14.2 g). Fruit quality in terms of TSS (14.2⁰ B), anthocyanin content (125.4mg/100 g), ascorbic acid content (107.5mg/100 g) and total sugars (8.38 %) was highest in Ofra followed by Chandler.

Sharma and Thakur (2008) evaluated 15 strawberry varieties in mid hills of Himachal Pradesh. They studied fruit yield and quality characters. They found that cultivar Chandler exhibited maximum total sugars (6.81 %). The cultivar Pajaro exhibited highest TSS and TSS/acid ratio. Highest acidity was found in Catskill while sugar/acid ratio was found highest in Selva. The cultivars Chandler and Selva were found to be most promising.

Singh et al. (2011) studied genetic variability in strawberry. The objectives of the study were to determine variability and inheritance of antioxidants, to identify antioxidant rich and productive genotypes and to suggest suitable breeding approaches. The genotypes, namely Ofra, Chandler, Festival and Camarosa showed higher concentrations of dietary antioxidants and therefore could be useful in future breeding. Results indicate that the effect of the genotypes on antioxidant contents is stronger than that of the environment. The high heritability (>80 %) and low genetic advance as percentage of mean (<40 %) for ascorbic acid and β -carotene contents respectively could be improved by heterosis breeding. However, selection and hybridization would be effective tools to enhance the phenols and anthocyanin content and yield potential as these traits showed high heritability (>80 %) and high genetic advance as percentage of mean (>40 %).

Singh et al. (2013) evaluated 22 genotypes to assess the nature and magnitude of variability in strawberry from diverse eco-geographic origins using Principal Component Analysis (PCA) and

single linkage cluster analysis (SLCA) assessing the divergence and similarity. The genotypes were classified into five groups for the determination of variability and four cluster groups for similarity by PCA and SLCA respectively. The highest inter-cluster distance was observed between cluster II and V (129.39) followed by IV and V (114.082) and the lowest between II and IV followed by III and IV. The highest intra-cluster distance was observed for cluster III and the lowest for the cluster VI and V. PCA showed that four principal component axes had Eigen values greater than one and altogether accounted for 77.34% of the total variation. The first two accounted for 57.88 % with PCA 1 accounting for 36.31 % and PCA 2 accounting for 21.57 %. The major contributing traits in PCA 1 was number of flowers, number of leaves and number of fruit/plant of leaflets per plant whereas in PCA 2, fruit length and fruit weight were major contributors for higher yield and quality. Thus, PCA was useful tool for identifying the characters responsible for major variability and to be used for breeding programme for higher yield and yield attributing traits whereas SCLA proved to be a better tool in multivariate analysis since it provided much clearing information concerning the extent of relationship among the genotypes.

Haque *et al.* (2015) studied 18 tissue culture variants of strawberry. The genotypes were grouped into three clusters based on Euclidean distance following Ward's method and highest intra-cluster distance was found in cluster III and inter-cluster distance was observed between genotypes of cluster I and III. It was observed that inter-cluster distance was always higher than those of intra-cluster distance. The maximum inter-cluster distance was observed between genotypes of cluster I and III (0.78) followed by clusters II and III (0.65).

Haque *et al.* (2015) studied 18 tissue culture variants of strawberry. Significant variation among the variants was found for all the characters. It was found that yield per plant was positively correlated with petiole length, days to opening of first flower, number of fruits per plant, % brix and weight of individual fruit. Path analysis revealed that petiole length, crown spread per plant, pollen sterility, number of fruits per plant, ascorbic acid content of individual fruit and weight of individual fruit had positive direct effects on fruit yield per plant.

PROPOSED RESEARCH OBJECTIVE

Considering the significance of biodiversity in strawberry and its evaluation under different agroclimatic condition the studies has been conducted with following set of objectives:

- i. To evaluate the performance of various genotypes of strawberry under polytunnel and shade net.
- ii. To study variability and heritability of various qualitative and quantitative traits of strawberry.
- iii. To determine the interrelationship among yield and yield contributing characters.

MATERIALS AND METHODOLOGIES

The study will be initiated in Month of September in Poly tunnel and Shade Net at Agricultural Farm, School of Agriculture, Lovely Professional University, Phagwara, Punjab. The details of materials used and methodology adopted has be described below:

1. Preparation of Raised bed for planting.
2. Selection of genotypes of strawberry: The genotypes mentioned below will be taken from NBPGR (National Bureau of Plant Genetic Resources), Division of Plant Exploration & Germplasm Collection). The following germplasms has been identified for evaluation.

S. No.	Germplasm code	Variety name	No. of plants
1	V ₁	Winter Dawn	30
2	V ₂	Shani	30
3	V ₃	E1-13#31	30
4	V ₄	E1-13#33	30
5	V ₅	FL-09-127	30
6	V ₆	E1-13#32	30
7	V ₇	E-22	30
8	V ₈	Sweet Charlie	30
9	V ₉	Yasmin	30
10	V ₁₀	Haidar	30
11	V ₁₁	Chandler	30
12	V ₁₂	Camarosa	30

3. **Treatments** : 12 genotypes
4. **Number of plants** :30 (10 in each replication)
5. **Spacing** :30 x 30cm

Observations to be recorded:

S. No.	Vegetative Parameters	S. No.	Fruit morphology and yield parameters
1	Plant height (cm)	1	Fruit shape
2	Plant spread (cm)	2	Fruit length (mm)
3	Number of leaves	3	Fruit breadth (mm)

4	Leaf area (cm ²)	4	Number of calyx per fruit
5	Number of runners per plant	5	Calyx attachment
6	Days to runner formation after planting	6	Altitude of sepals
7	Leaf base shape	7	Core colour
8	Leaf apex	8	Fruit colour
9	Leaf blistering	9	Evenness of colour
10	Nature of leaf	10	Evenness of surface
11	Leaf margins	11	Achene placement
12	Petiole length	12	Width of band without achenes (mm)
13	Chlorophyll content	13	Days to maturity
S. No.	Flowering Parameters	14	Number of fruits per plant
1	Flower diameter (mm)	15	Average berry weight (g)
2	Flower type	16	Yield per plant (g)
3	Petal arrangement	17	Total marketable yield (g)
4	Petal size (mm)	S. No.	Fruit quality parameters
5	Petal colour	1	Total soluble solids (^o Brix)
6	Number of petals	2	Acidity (%)
7	Number of stamens	3	Antioxidants
8	Anther attachment	4	Reducing sugars (%)
9	Days to flowering	5	Non-reducing sugars (%)
10	Opening of first flower	6	Total sugars (%)
11	Opening of last flower	7	TSS/acid ratio
12	Duration of flowering	8	Sugar/acid ratio

Morphological characterization in strawberry involves recording variation in habit, leaf, flower, and fruit traits (Dale 1996). Morphological characters traditionally identified crop species and varieties (Nielsen and Lovell 2000) and have been used in Argentina to certify cultivar identity in strawberry (Garcia et al. 2002). In the United States and Europe, morphological markers are used in addition to isozyme markers in plant patent descriptions (Nielsen and Lovell 2000). The current study also includes evaluation on the basis of certain molecular parameters and will cover following steps:

- i. DNA extraction
- ii. Amplification with selected markers (PCR analysis)
- iii. Genetic variability among selected cultivars

The study will be conducted by using following markers:

ARSFL_9	F: GCGAGGCGATCATGGAGAGA
	R: GCGTTTCCTACGTCCCAATAAATC
ARSFL_10	F: GCGTCAGCCGTAGTGATGTAGCAG
	R: GCGCCAGCCCCTCAAATATC
ARSFL_11	F: GCGAAGCATAACTGGCAGTATCTG
	R: GCGGGCCTAGGTGATCTTGGA

Proposed Research Methodology

- **Plant height (cm):** The height of plant will be recorded using measuring tape from the crown level to apex of the primary leaf. It will be measured in centimeters (cm).
- **Plant spread (cm):** The spread of the plant will be measured in North-South (N-S) as well as in East West (E-W) direction.
- **Number of leaves:** The number of leaves can be counted at the end of harvesting and the mean is expressed as the number of leaves per plant.
- **Leaf area:** The leaf area index will be measured by using leaf area meter.
- **Number of runners per plant:** The number of runners per plant can be counted at the end of harvesting.
- **Leaf base shape:** The base shape will be recorded according to the guidelines of UPOV (2012). It can be acute, obtuse or rounded.
- **Leaf blistering:** The base blistering will be recorded according to the guidelines of UPOV (2012). It can be absent or weak, medium or strong.
- **Nature of leaf:** The leaf of strawberry is compound.
- **Leaf margins:** The base margins will be recorded according to the guidelines of UPOV (2012). It can be serrate to crenate.
- **Petiole length:** The petiole length will be measured in centimeters (cm) using measuring tape.
- **Days to runner formation after planting:** The days to runner formation will be recorded from day of planting to the day of first runner formation.
- **Days to fruit maturity from flowering:** The flowers will be tagged as they will open and days to fruit maturity will be worked out.

- **Number of fruits per plant:**The number of fruits per plant will be counted at the time of fruit maturity and the result was expressed as number of fruits per plant.
- **Petal arrangement:** The petal arrangement will be recorded according to the guidelines of UPOV(2012). The petal arrangement can be overlapping, touching or free.
- **Fruit length (mm):** The length of fruit will be measured from calyx end to the pointed end or apex of the berries with the help of vernier caliper and expressed in mm.
- **Fruit breadth (mm):** The diameter of berries will be measured from the shoulders of the berries with the help of vernier calliper and expressed in mm.
- **Calyx attachment:** The calyx attachment position will be recorded according to the guidelines of UPOV(2012). It can be inserted, level with the fruit or raised.
- **Altitude of sepals:** This will be recorded according to the guidelines of UPOV (2012). It can be upwards, downwards or outwards.
- **Fruit color:**This will be recorded according to the guidelines of UPOV (2012). The color of the flesh excluding the core will be observed.
- **Core color:** This will be recorded according to the guidelines of UPOV (2012). The core color is observed excluding the flesh.
- **Evenness of color:**This will be recorded according to the guidelines of UPOV (2012). The color can be strongly uneven to slightly uneven or even.
- **Evenness of surface:**This will be recorded according to the guidelines of UPOV (2012). This can range from strongly uneven to slightly uneven or even.
- **Achene placement:**This will be recorded according to the guidelines of UPOV(2012). It can be below the surface, level with the surface or above the surface of the fruit.
- **Width of band without achenes:** This will be recorded according to the guidelines of UPOV(2012). It can range from absent or very narrow to broad or very broad.
- **Average berry weight (g):**Ten randomly selected fruits from each plot will be used for measuring the fruit weight. The weight will be measured on electronic balance and the average berry weight will be calculated and expressed in grams (g).
- **Yield per plant (g):**The weight of entire fruits harvested per plant will be recorded for each cultivar and the result will be expressed as yield per plant.

- **Yield per hectare (tonnes):**The weight of entire fruits harvested from each plot will be recorded for each cultivar. The yield per hectare was worked out and expressed as tonnes per hectare.
- **Total soluble solids (°B):**The TSS of ripe fruit juice will be determined with the help of a hand refractometer (0-32 °brix) by putting a few drops of juice on the prism. The refractometer will be calibrated with distilled water before use. The total soluble solids are expressed in °B.
- **Titrateable acidity:**A known weight of the fruit sample will be crushed and taken in a 100 ml volumetric flask and the volume will be made up to 100ml by adding distilled water. After filtration, 10 ml of the filtrate will be taken in a separate conical flask and titrated against 0.1 N (4g/1000g)sodium hydroxide using phenolphthalein as an indicator. The end point will be determined by the appearance of a faint pink colour. Note the readings and calculate using the formula.
- **Total soluble solids/acid ratio:**It will be worked out by dividing the TSS totitrable acidity.
- **Reducing sugars:** Take 1ml of strawberry juice and make the volume to 3ml by adding distilled water. Add 3ml of the DNS reagent. Keep in water bath at 100 degrees centigrade for five minutes. Afterwards add 1ml of 40% Rochelle salt. Allow it to cool and take O.D.at 510nm.
- **Total sugars:** 4ml of anthrone reagent will be added to 1ml of juice. Water bath at 100 degrees centigrade for 8 minutes and check the O.D. at 630nm.
- **Non-reducing sugars:** It will be calculated by subtracting reducing sugars from total sugars.
- **Sugar/acid ratio:** It will be determined by dividing the total sugars to acid content.

Statistical analysis

The statistical analysis will be carried out for each observed character by using MS-Excel, OPSTAT and SPAR 1.0 packages. The mean values of data will be subjected to analysis of variance as described by Gomez and Gomez (1983) for Randomized Complete Block Design. For estimation of different statistical parameters, following procedure will be adopted:

1. Analysis of variance

2. All the traits, which will differ significantly, will be utilized for estimation of following genetic parameters:
- a) Mean performance and genetic variability
 - b) Heritability (in broad sense)
 - c) Genetic advance (GA)
 - d) Genetic gain (GG)
 - e) Correlation coefficients
 - f) Path analysis
 - g) Genetic divergence (D^2 analysis)

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