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Research Article

# DISTINCTNESS, UNIFORMITY, AND STABILITY TESTING OF COTTON (*Gossypium hirsutum* L) GERMPLASM LINES

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**Abstract:** The experiment was conducted at Chaudhary Charan Singh Haryana Agricultural University, Hisar for two consecutive kharif seasons *i.e.*, 2018-19 and 2019-20. The experimental material consisted of 150 germplasm lines of *Gossypium hirsut*um L. planted in augmented design with three checks (H1098i, H1226, and H1236). The categorization of germplasm lines based on DUS traits was done following all the guidelines by PPVFRA. A total of 34 traits were studied for the same and ample variation was seen among genotypes for hypocotyl pigmentation, pubescence on stem, stem pigmentation, leaf color, pigmentation of petiole, flower petal color, boll shape, prominence of tip, plant height, growth habit, seed index, ginning percentage, fiber length, fiber strength and fiber fineness. Characters like leaf shape, leaf hairiness, the position of stigma, pollen color, boll color, boll opening, boll weight, seed fuzz density and fiber maturity were seen less varied among the genotypes whereas traits *viz*. leaf appearance, presence of gossypol glands and leaf nectaries, bract type, anther filament coloration, boll bearing habit, boll surface, fiber color and seed fuzz color did not show any variation. All the qualitative traits were stable and uniform in both seasons, quantitative traits showed some variation as they have more environmental effects.

**Keywords:** *Cotton, Germplasm lines, DUS traits, PPVFRA, Variation*

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## Introduction

Cotton is the major fiber crop, also known as "white gold". It serves as raw material for the textile industry and earns a good foreign exchange. India is a leading country in terms of area and raw cotton production. The area under cotton cultivation in India is about 129.57 lakh hectare with a production of 371 lakh bales and the productivity of 487kh/ha. Major Cotton producing states are Maharashtra (26.63%), Gujarat (17.96%), Andhra Pradesh (13.75%) and Madhya Pradesh. In Haryana, cotton is grown in an area of 7.01 lakh hectare with a production of 22 lakh bales and productivity of 502 kg/ha lint.

Cotton belongs to family Malvaceae and genus Gossypium. There are four cultivable varieties among which *G.hirsutum* (2n=52) occupies 90% of the net sown area under cotton crop. Repeated use of several superior inbreds in breeding programs, genetic diversity has reduced greatly which has led to massive genetic erosion over the last decade [1]. Therefore, plant breeders need to put attention on the characterization and conservation of cotton germplasm. PPVFR, Act 2001facilitates the protection of plant varieties and farmers' rights. It provides DUS (distinctness, uniformity and stability) testing guidelines and traits to be examined which are necessary to protect, register, characterize and identify a plant variety or cataloging of germplasm line. DUS characterization aids in crop improvement by selection of better parents with specific trait to be used in the breeding programme. A total of 37 DUS characters are enlisted by PPVFR for tetraploid cotton. In present investigation we have analyzed 34 different morphological markers using DUS testing manual of protection of plant variety and farmers' rights authority, India.

## Materials and methods

The experiment was comprised of one hundred fifty germplasm lines (GCW1-GCW150) of upland cotton *Gossypium hirsutum* grown in kharif season of 2018 - 19 and 2019-20 at Research area of Department of Genetics and Plant Breeding CCSHAU, Hisar, 125001.

Augmented design was followed with three check varieties *i.e.* H1098i, H1226 and H1236 and 15 genotypes randomly arranged in a block. The length of the row was 6m, distance between rows was 1.35m and distance between plants was maintained at 30cm. Data on five randomly selected plants from each genotype was recorded for various quantitative and qualitative characters mentioned in [Table-1].

Table-1 *Phenotypic traits recorded on field of 150 germplasm lines*

| SN | Traits | SN | Traits |
|---|---|---|---|
| 1 | Hypocotyl pigmentation | 18 | Anther filament coloration |
| 2 | Leaf color | 19 | Pollen color |
| 3 | Leaf hairiness | 20 | Boll bearing habit |
| 4 | Leaf appearance | 21 | Boll color |
| 5 | Leaf gossypol glands | 22 | Boll shape |
| 6 | Leaf nectaries | 23 | Boll surface |
| 7 | Leaf petiole pigmentation | 24 | Prominence of tip in boll |
| 8 | Leaf shape | 25 | Boll opening |
| 9 | Plant stem hairiness | 26 | Boll weight (g) |
| 10 | Plant stem pigmentation | 27 | Seed fuzz color |
| 11 | Plant height (cm) | 28 | Seed index |
| 12 | Plant growth habit | 29 | Ginning out turn % |
| 13 | Bract type | 30 | Fiber color |
| 14 | Days to first flower | 31 | Fiber length (2.5% span length) (mm) |
| 15 | Flower petal color | 32 | Fiber strength (g/tex) |
| 16 | Flower petal spot | 33 | Fiber fineness (micronaire) value) |
| 17 | Flower stigma position | 34 | Fiber maturity (%) |

## Result and discussion

### Seedling stage

Hypocotyl pigmentation is observed at seedling stage after 5-6 days of sowing. Hypocotyl pigmentation is an important morphological marker which is easy to observe as it is governed by a single dominant gene.

It was categorized as green hypocotyl (pigmentation absent, scoring 1) and purple hypocotyl (scoring 9) in which anthocyanin coloration was present [Fig-1]. In present investigation 34 genotypes showed presence of hypocotyl pigmentation and 116 exhibited absence of hypocotyl pigmentation. Similar categorization was done by Ponnuswamy *et al.* (2003) [2], Pooja *et al.* (2016) [3], and Reddy *et al.* (2007)[4].

Fig-1 Shows pigmented and non-pigmented hypocotyl

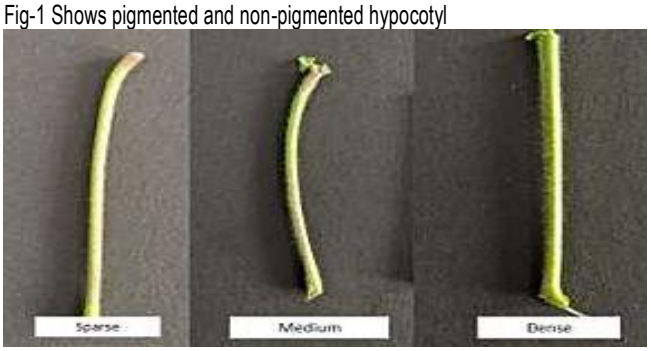


Fig-2 Presence of gossypol glands and nectaries at the back of leaf

Fig-3 Shows different leaf color A: Green, B: Light green and C: Dark red

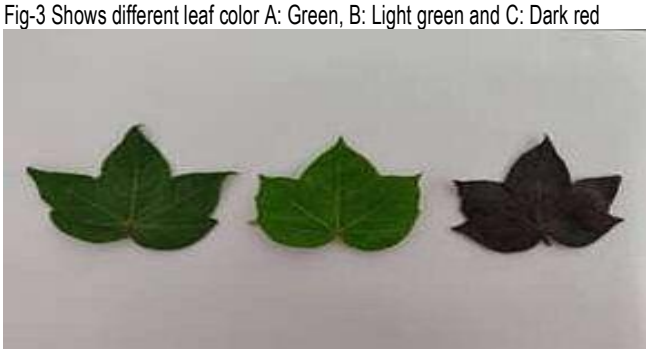

Fig-4 Different leaf shapes of cotton, palmate, semi-digitate and digitate

Fig-5 Petiole pigmentation present and absent


Fig-6 Stem pigmentation present in A and absent in B

Fig-7 Shows hair density on stem of cotton plant

Fig-8 Shows normal type bract

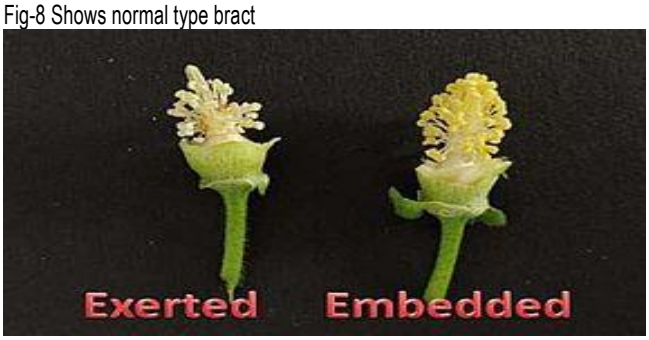


Fig-9 Depicts stigma position in the flower

Fig-10 A. shows absence of petal spot, B. shows presence of petal spot

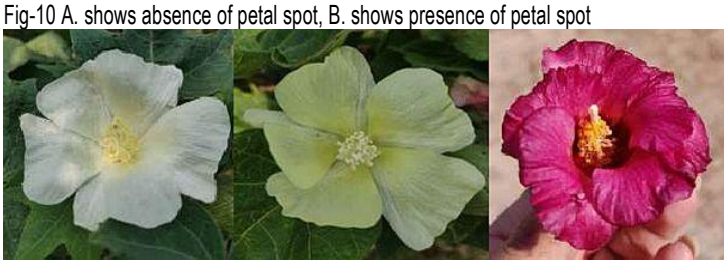

Fig-11 Flower petal color variation; a. cream, b. yellow, c. purple

Fig-12 Yellow and Cream pollen color is shown in the plate

Fig-13 Shows boll surface A. pitted and B. smooth

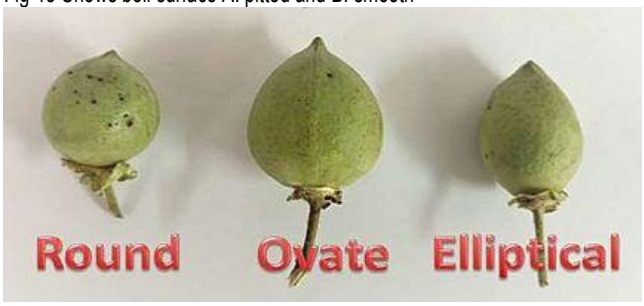


Fig-14 Different boll shape present in genotypes

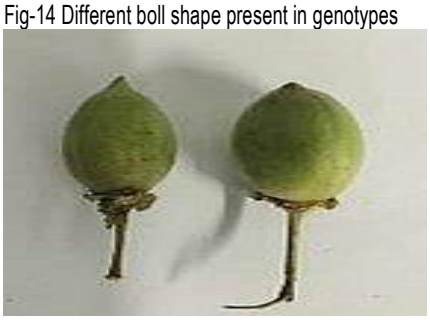

Fig-15 Prominence of tip in boll can be seen in the plate

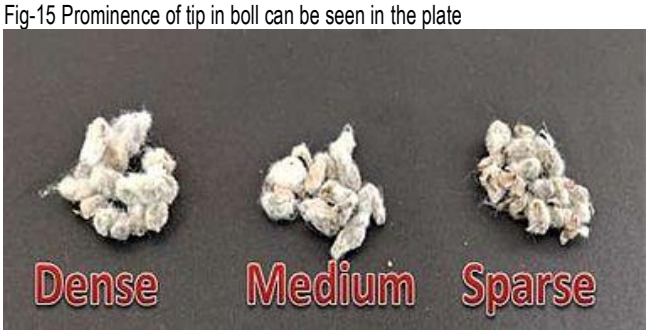


Fig-16 Shows seed fuzz density as dense, medium and sparse

Fig-17 A pictorial view showing difference in fiber length of seed cotton

**Square formation**

Square is a fruiting bud, this stage comes almost after 1 month of sowing of crop. At this stage stem hairiness has to be noted. Density of hair on the stem [Fig-2] is categorized in four groups; in present study 26 germpalsm lines had sparse hair in stem (scoring 3), 103 possessed medium hair (scoring 5) and 21 lines had dense hair on stem (scoring 7). No genotype was found with smooth stem or complete absence of hair on stem (scoring 1). Many have characterized genotypes on the basis of stem hairiness some of them are Aruna *et al.* (2012) [5], Pooja *et al.* (2016) and Sangwan *et al.* (2016) [6].

**Fifty percent (50%) flowering stage**

Generally, crop attains this stage from 50-60 days after sowing where at least one flower is open in 50% of the plant population. All the leaf and flower characters are examined at this stage.

Leaf characters: There are four types of leaf shape in tetraploid cotton *viz.* palmate (1), semi-digitate (2), digitate (3) and laceolate (4). Leaf shape can affect photosynthetic rate, water use efficiency, yield and boll rot resistance which makes it an important trait to include in identification. The multiple alleles responsible for different leaf shapes belongs to single incomplete dominant locus L-D on chromosome 15-D1 [7]. Out of 150 genotypes 147 had palmate type of leaf, 2 genotypes (GCW61, GCW117) had semi-digitate type (semi okra) and GCW133 possessed digitate type (okra type) leaf [Fig-3].

On the basis of leaf color genotypes were categorized as light green (1), green (2) and dark red (4). Forty nine genotypes were having light green color leaf and 100 genotypes had green color leaf. Only one genotype GCW131 had dark red leaves which is shown in [Fig-4]. The appearance of leaf may be flat (2) or cup (1) like due to upward curling. All 150 genotypes examined exhibited flat leaf appearance in present studies. The pubescence of leaf is classified as sparse (1), medium (5) and dense (9). Ninetyseven genotypes showed sparse hair on leaf blade whereas 53 genotypes showed medium hair presence. No genotype was found with dense hair on the leaf. Leaf pubescence is associated with light reflectance and insect resistance; hence it is a vital trait to study. Two pair of genes HP1 and HA2 play role in pubescence of leaf in cotton, which shows additive effects when present in dominant form [8].

A cup shaped small notch present on leaf mid rib and major vein at the back of leaf is nectaries it secretes sweet gummy fluid to attract insects whereas gossypol glands secretes a phenolic compound which is toxic to non-ruminants. All 150 genotypes showed the presence of gossypol glands and leaf nectraries and scored as 9 [Fig-5], therefore no variation was found for these two traits in the studied genotypes. The leaf characteristics were also studied by Aruna *et al.* (2012), Padmavathi *et al.* (2009) [9], Pooja *et al.* (2016), Reddy *et al.* (2007 a), Sagar *et al.* (2019) [10], and Sangwan *et al.* (2016) and concluded that characters like leaf hairiness, leaf color, leaf size are stable and uniform in performance thus act as useful diagnostic characters for identification. Whereas Anjani *et al.* (2018) [11] observes that leaf shape, leaf color, leaf hairiness, gossypol glands and nectraies does not show much variation among the tested genotypes.

Anthocyanin pigmentation may be found on leaf petiole and stem of the cotton plant. The Presence (9) or absence (1) of pigmentation in petiole was recorded and 55 genotypes had pigmented petiole whereas remaining 95 had green petiole [Fig-6]. Red color pigmentation on stem was present in 36 genotypes whereas remaining 114 possessed green stem [Fig-7]. Grouping of genotypes on the basis of pigmentation on petiole and stem is in conformity with the results of Aruna *et al.* (2012), Padmavathi *et al.* (2009), Pooja *et al.* (2016) and Sangwan *et al.* (2016).

Flower characters: Bracts can be of two shapes i.e., normal (3) and frego (5). In our experimental material no variation was found for bract type as all the genotypes had normal type of bracts [Fig-8]. On the basis of petal color [Fig-9]; out of 150 germplasm lines 128 had cream petals (1) and 17 with yellow petals (2). Only 5 genotypes (GCW10 GCW11 GCW12 GCW14 and GCW122) possessed deep yellow petal flower (3). The stigma position in flower may be embedded (3) or exerted (5) shown in [Fig-10] and it was found exerted in 143 genotypes, embedded in 7 genotypes. Petal spot is a dark inner circle of anthocyanin coloration at the base of petal [Fig-11]; this trait is scored as present (9) or absent (1). It is used as morphological marker by breeders and seed producers.

Petal spot was absent in all genotypes. Purple coloration on stalk of the androceum was also recorded and all the genotypes showed absence (1) of coloration on anther filament. Pollen can be of white (1), cream (2), yellow (3), deep yellow (4) or of purple color (5). In our experiment 141 genotypes were observed with cream pollens and remaining 9 genotypes with yellow pollens [Fig-12]. Floral traits are very important descriptors and are easy to score, they have been used by Aruna *et al.* (2012), Padmavathi *et al.* (2009), Pooja *et al.* (2016), Reddy *et al.* (2007), Sagar *et al.* (2019) and Sangwan *et al.* (2016) for identification and characterization of different genotypes.

**Boll bursting stage**

Boll related characters are observed at boll bursting stage where a boll or capsule shows cracks on walls. Boll bearing habit can be solitary (1), when one boll is present on one tertiary branch or in cluster (9). In entire 150 genotypes solitary boll bearing habit was found. The boll color is categorized as green (3) or red (5). All the germplasm lines except GCW131 had green boll color in the investigation; only GCW131 possessed red boll color. Boll surface can be smooth (1) or pitted (9) can be seen in [Fig-13], no variation was found for this character it was found smooth in all the tested genotypes. Moving to the next trait that is boll shape; cotton boll can be of 3 shapes round (3), ovate (5) and elliptical (7). Only 3 genotypes (GCW6 GCW17 and GCW46) were found with round boll and 100 exhibited ovate boll shape. Fortyseven genotypes were there with elliptical boll shape [Fig-14]. Tip prominence at the end is categorized as blunt (absence, 1) or pointed (presence, 9). Fortyone germplasm lines had blunt end and pointed tip bolls were found in 109 germplam lines [Fig-15]. Genotypes reveal a great variation in boll shape and tip prominence hence these two traits are very important discriptors. Grouping on basis of boll shape, color, surface and tip prominence is also performed by Aruna *et al.* (2012), Padmavathi *et al.* (2009), Pooja *et al.* (2016), Reddy *et al.* (2007) and Sangwan *et al.* (2016).

**First picking**

At least 20% of bolls in each plant must have opened at this stage. Traits like plant height, growth habit, boll opening, boll weight etc. are recorded at this time. On the basis of plant height 2 genotypes (GCW17 and GCW36) were grouped under medium tall category (91-120 cm, scoring 5), 41 genotypes under tall category (120-150 cm, scoring 7) and 107 under very tall category (>150 cm, scoring 9). Hamidi *et al.* (2018) [12] and Balakrishnan *et al.* (2020) [13] were also able to distinguish between genotypes on the basis of plant height. Growth habit is classified as zero branching (1), compact (3), semi-spreading (5) and spreading (7). Spreading plant growth habit having canopy diameter more than 60 cm was exhibited by 71 genotypes, semi-spreading type with 31-60 cm canopy diameter in again 71 genotypes and compact type with 30cm canopy diameter was found in 8 genotypes among all the examined entries. Plant height and growth habits are observed towards the end period of crop because plant gets their full growth by this time. In this investigation 147 genotypes out of 150 had open type (5) boll opening whereas 3 had semi-open type (3). Anjani *et al.* (2018) and Rathinavel *et al.* (2019) [14] also reported that genotypes do not have much variation for boll opening. Fiber color of all the genotypes was observed as white (1) and no other coloration was seen. Whereas on the basis of boll weight 99 genotypes possessed less than 3g of weight per boll (scoring 1). Fortynine genotypes had boll weight between 3-4g (scoring 3) and come under small category. Only two genotypes (GCW7 and GCW66) had boll weight between 4.1-5g (scoring 5) and no genotype had large or very large boll size. Begum and Hossain (2011) [15] were able to find out a superior genotype mainly due to its high seed cotton yield and average weight of seed cotton per ten bolls; hence boll weight is a vital character for identification and grouping of genotypes. Aruna *et al.* (2012), Padmavathi *et al.* (2009), Pooja *et al.* (2016) and Sangwan *et al.* (2016) also studied these traits in different genotypes and were able to characterize them.

**Final harvest**

After the crop is finally harvested in October month, seed and fiber quality parameters are examined. In present study seed fuzz color was white (1) in all the genotypes whereas its density differed as 133 genotypes showed medium fuzz density (5) and other 17 showed dense fuzz density (7), refer [Fig-16]. Seed fuzz is the fiber threads which remain on seed coat after ginning. Seed index or hundred seed weight is divided into various groups having weight between 5g to 11g. Out of 150 genotypes 3 (GCW78, GCW90, GCW129) fell into very small group (1) in which weight recorded were less than 5g, 121 in small group weigh between 5-7g (3) and 26 under medium group with weight range 7-9g (5). No genotypes were found with bold (7) or very bold type (9) seeds. Variation in 100 seed weight may be due to inherent properties that were present during the period of crop growth, seed development and maturation. Embryo size and other food reserve materials with respect to genotypes can also alter seed weight and generate variation. Balakrishnan *et al.* (2020) Reddy *et al.* (2007a) examined genotypes based on 100 seed weight. Ginning percentage is the amount of fiber extracted from seed cotton yield after ginning. GCW32 and GCW84 came under low category (3) with ginning percentage being between 31-32%, GCW5, GCW73, GCW110, GCW139, and GCW150 these 5 genotypes were having medium ginning percentage (5) values (33-34%), 20 genotypes fell under high group (7) where ginning percentage ranged between 35-36% and maximum genotypes *i.e.* 123 were having ginning percentage more than 36 percent (9) thus categorized under very high group.

**Fiber quality traits**

Fiber quality traits are important to estimate as the major product of cotton crop is its fiber. Cotton is the second most developed sector in textile industry thus with high productivity and production, fiber quality also has to be maintained. Among the 150 examined genotypes, 24 genotypes had medium fiber length [Fig-17] with values being between 20.5-24.5 mm (3), 110 genotypes had medium long fiber length (25-27 mm, scoring 5) and 16 genotypes showed long fiber length (7) 27.5-32mm. fiber strength is the force required to break the fiber of unit linear density. On the basis of fiber strength GCW37, GCW98, GCW104, and GCW144 had medium fiber strength (5) ranging from 21-24 g/tex, 103 germplasm lines were having strong fiber (25-28 g/tex, scoring 7) and 43 genotypes had very strong fiber (9) which was more than 29 g/tex. Fiber maturity is another index for fiber quality in which development of fiber walls are assessed. Out of 150 genotypes 114 fell into good category (7) with values being between 75-80% and 36 into very good category (9) with values more than 81%. Observations recorded for fiber fineness revealed that GCW23, GCW58, GCW114, GCW118, and GCW120 had coarse (5-5.9 µ/inch) type of fiber (3), 139 genotypes had medium fineness (4.0-4.9 µ/inch, scoring 5) whereas 6 genotypes were having fine type of fiber (3.0-3.9 µ/inch, scoring 7). On the basis of fiber quality parameters GCW1, GCW2, GCW4, GCW12, GCW18, GCW28, GCW69, GCW135 and GCW143 are the best genotypes with long and strong fiber, good maturity and medium fineness. Balakrishna *et al.* (2016) [16] studied 7 lint descriptors and was able to identify good fiber quality genotypes which had potential to be used as parents in further hybridization programme. Thus fiber quality parameters are very important descriptors. Iqbal *et al.* (2015) [17] characterized 26 accessions of Gossypium arboreum on basis of fiber quality traits.

This investigation suggests genotypes could be distinguished on the bases of these 34 characters thus they possess distinctness. Traits were found stable and uniform through both the seasons hence fulfills the DUS testing requirements. Further selection for any specific trait can be performed from these germplasm lines on the basis of data collected [18].

**Conclusion**

All the qualitative traits were stable and uniform in both seasons, quantitative traits showed some variation as they have more environmental effects. For example Characters like leaf shape, leaf hairiness, the position of stigma, pollen color, boll color, boll opening, boll weight, seed fuzz density and fiber maturity were seen less varied among the genotypes whereas traits *viz*. leaf appearance, presence of gossypol glands and leaf nectaries, bract type, anther filament coloration, boll bearing habit, boll surface, fiber color and seed fuzz color did not show any variation. This shows the stability of different traits and they are helpful for characterizing any germplasm lines. It helps access the genetic variability between different lines and use them in future breeding programs.

**Application of research:** Genetic variability and characterization of germplasm lines is one of the most important prerequisite in plant breeding it helps us to choose better parents for future programs.

**Research Category:** Genetics and Plant Breeding

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**Author Contributions:** All authors equally contributed

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**Study area / Sample Collection:** CCSHAU, Hisar, 125001, India

**Cultivar / Variety / Breed name:** Cotton (*Gossypium hirsutum* L)

**Conflict of Interest:** None declared

**Ethical approval:** This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

**References**

[1] Meredith Jr W.R. (2000) *Better Crops*, 84(4), 6-9.
[2] Ponnuswamy A.S., Bhaskaran M. and Sastri G. (2003) *Training manual, Variety characterization by Image Analysis and Electrophoresis,* 106-120.
[3] Pooja R., Sangwan O., Siwach S.S., Sangwan R.S., Pundir S.R. and Nimbal S. (2016) *Journal of Cotton Research and Development*, 30(1), 36-40.
[4] Reddy Manjunath, Hunje Ravi, Biradar D.P. and Vyakarnahal B.S. (2007) *Karnataka Journal of Agriculture Science*, 20(3), 511-513.
[5] Aruna P., Rao P.S., Anuradha G. and Keshavulu K. (2012) *Journal of Research ANGRAU*, 40(3), 113-115.
[6] Sangwan O., Pundir S.R. and Mandhania S. (2016) *International Journal of Food, Agriculture and Veterinary Sciences,* 6 (2), 22-26.
[7] Andres R.J., Bowman D.T., Jones D.C. and Kuraparthy V. (2016) *The Journal of Cotton Science*, 20, 330-340.
[8] Muttuthamby S., Aslam M. and Khan M.A. (1969) *Euphytica*, 18, 435-439.
[9] Padmavathi A., Ahmed M.L., Ramakumar P.V. and Anilkumar P. (2009) *The Andhra Agricultural Journal*, 56 (2), 186-191.
[10] Sagar S.N., Sangwan R.S., Kumar P., Jangid K. and Reddy B. (2019) *Journal of Pharmacognosy and Phytochemistry*, 8(5), 1100-1103.
[11] Anjani A., Padma V., Ramana J.V. and Satish Y. (2018) *International Journal of Current Microbiology and Applied Science*, 7(6), 3900-3904.
[12] Hamidi A., Bazdi G. and Jafari Y. (2018) *Journal of Crop Breeding*, 10(27), 66-74.
[13] Balakrishnan T., Vennila S., Saravanan, K.R., and Karthikeyan P. (2020) *Plant Archives*, 20(1), 3606-3608.
[14] Rathinavel K. (2019) *International Journal of Current Microbiology and Applied Science*, 8(2), 2039-2057.
[15] Begum S. and Hossain S. (2011) *SAARC Journal of Agriculture*, 9, 1-12.
[16] Balakrishna B., Reddy V.C. and Reddy K.V.S. (2016) *Environment and Ecology,* 34(1A), 262-266.
[17] Iqbal M.A., Ammad A. and Zafar Y. (2015) *Pakistan Journal of Botany*, 47(6), 2347-2354.
[18] Patil R.B. and Suryawanshi Y.B. (1996) *NSP (crops) Bull. Venus Publishers. New Delhi*.