



Visually assessable morphological descriptors-based establishment of distinctiveness, uniformity and stability of tetraploid cotton (*Gossypium* spp.)

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ABSTRACT : The development of crop varieties with enhanced economic value requires considerable resources, time and intellectual input. Seeking legal rights on crop varieties is the best way to protect them from unlawful marketing of the seeds other than their breeders/developing agencies for deriving economic benefits from them. Seeking legal protection and commercialization of crop varieties under Indian PPV and FR Act, 2001 requires establishment of their distinctness (D), uniformity (U) and stability (S). DUS of crop varieties are established predominantly using morphological descriptors. Therefore, characterizations of genotypes are essential for their protection. The guidelines for the conduct of the DUS test for tetraploid and diploid cottons were finalized and published in 2008. In tetraploid guideline 37 traits, including 22 essential (genetically controlled) and 15 optional (environmentally influenced) traits were fixed. With this perspective, 54 released varieties of cotton were characterized following the guidelines to form the primary database for the selection of appropriate reference varieties during the conduct of DUS testing of new candidate varieties. The frequencies for expression of different states of all characteristics following the guidelines are drawn. The range of variability documented in this study would be valuable on the context of breeding a new variety with distinctiveness.

Keywords: Distinctiveness, frequency distribution, *Gossypium*, notified varieties, novelty, PPV and FRA, stability uniformity

Intellectual property (IP) is the foundation of knowledge-based economy. It pervades all sectors of economy and is increasingly becoming important for ensuring competitiveness of the enterprises. Depending on the nature and tangibility of the intellectual property, different type of rights such as patent, copy rights, trademarks, industrial designs, plant breeders or farmers rights, protection of undisclosed information, protection of database etc., are granted by the respective competent authority. India being the signatory and founding member of world trade organization; thrust on plant variety protection has been envisaged under the provisions of Trade Related aspects of Intellectual Property Rights (TRIPS), which is an integral part of WTO. The member countries of WTO have freedom in formulating their own system of plant variety protection either patent or an effective sui generis system under the

provisions of article 27.3 (b) of TRIPS agreement.

Accordingly, India opted for the sui generis system of plant variety protection that paved the way for enactment of Protection of Plant Varieties and Farmers Rights Act, 2001 (Anonymous., 2008) The section 14 of protection of plant varieties and farmers rights act provide immense opportunity for the registration of genera, species an extant variety, a farmers variety and a new plant variety provided it should confirm to the criteria of Novelty, Distinctiveness, Uniformity and Stability

Varieties of cotton crop are considered as intellectual properties as their breeding is based on sound genetic principles, selection skill and other knowledge resources. Though they are developed from genetic resources accumulated through natural evolution, they have desirable rearrangements of gene combinations which are manifested over morphological features with an

enhanced economic value. Thus, the development of crop varieties requires considerable resources, time and intellectual input (Shrikrishna and Ramesh, 2020). The individual farmers/agencies/institutions other than the breeder or his nominee can easily commercialize crop of protected varieties, however, seed production and marketing of them is prohibited to others, other than the breeder or his nominee as to safeguard the economic benefits to the originators of crop varieties. Seeking legal rights on crop varieties is the best and the only way to protect them from an unlawful claim of their origin/development and deriving economic benefits from them. Therefore, almost all the nations including India have enacted appropriate laws to protect the rights of originators of crop varieties. In India, granting legal rights on crop varieties under Protection of Plant Varieties and Farmers Rights Act, 2001 requires establishment of their distinctiveness (D), uniformity (U) and stability (S) based on field observation of essential morphological traits recorded in two locations consecutively for two similar seasons, trials conducted as per the protocol of the National Test Guidelines (<http://www.plantauthority.gov.in>). In the event of establishment of distinctiveness based on morphological traits is failure, the competent authority may consider physiological traits including disease and insect pest resistance as special character for establishing DUS (Pratibha *et al.*, 2004).

In India, the enactment of PPV and FR Act, 2001 was followed by the establishment of the Plant Varieties and Farmers Rights Authority (PPV and FRA) in 2005 for implementation of various provisions of the Act (Anonymous, 2008). Crops eligible for protection have been notified by the authority from time to time. Cotton crop including four species was notified on 31st December, 2007 (S.O.2229 (E)). This has led to characterization of cultivars, especially to maintain the identity of notified varieties and the parental lines of hybrids under Seeds Act, 1966

to serve as reference varieties while DUS testing of candidate varieties. With this objective the present study was conducted to establish morphological descriptors-based DUS of extant notified varieties as well advanced breeding line and released varieties.

MATERIALS AND METHODS

The present study was conducted at Central Institute for Cotton Research, Regional Station, Coimbatore from 2012 to 2015. In this study observations recorded on 54 tetraploid notified varieties planted as reference varieties in a DUS trial for comparison with new candidate variety. The experiment was conducted in a randomized block design, three replication, 12 rows/plots with 90 x 60 cm spacing. Recommended agronomic practices and prophylactic measures were adopted for raising a good crop. Observations on 36 morphometric traits were recorded on ten randomly selected plants per replications at phenological stages as per the DUS testing guidelines of tetraploid cotton (Anonymous, 2008).

The scores for hypocotyl pigmentation was given as 1- Absent, 9- Present; for leaf colour the rating given was 1- Light green, 2-Green, 3- Light red and 4-Dark red; Leaf Hairiness was scored as 1-sparse, 5- Medium, 9- Dense; Similarly Leaf Appearance: 1-Cup, 2-Flat; Leaf Gossypol Glands as 1- Absent, 9- Present; Leaf Nectaries: 1- Absent, 9- Present; Leaf petiole pigmentation: 1- Absent, 9- Present; Leaf shape: 1- Palmate, 2- Semi-digitate, 3-Digitate, 4- Lanceolate; Plant stem hairiness: 1- smooth, 3- sparse, 5- medium, 7-dense; plant stem pigmentation: 1-Absent, 2-Present; Bract type: 3-Normal, 5-Frego; Flower petal colour: 1- Cream, 2-Yellow, 3-Deep yellow, 4-purple; Flower petal spot: 1-Absent, 9-Present; Flower stigma: 3-Embedded, 5-Exerted; Flower anther filament colouration: 1-Absent, 9- Present; Flower pollen colour: 1-White, 2- Cream, 3-

Yellow, 4-Deep yellow, 5- Purple; Flower male sterility: 1-Absent, 9-Present; Boll bearing habit: 1- Solitary, 9- Cluster; Boll Colour: 3-Green, 5-Red; Boll shape 3-Round, 5-Ovate, 7-Elliptic; Boll Surface: 1-Smooth, 9-Pitted; Boll Prominence of tip: 1- Blunt, 9-Pointed; Boll opening: 3- Semi-open, 5- Open; Seed fuzz: 1-Naked, 3-Sparse, 5-Medium, 7-Dense; Seed fuzz colour: 1-White, 2-Grey, 3-Green, 4-Brown; Fibre colour: 1-White, 2-Cream, 3-Green, 4-Brown. Care has been taken not to record observations on plants in border rows. Leaf characteristics observed on the fourth fully expanded leaf from the top of the main stem at 50 per cent flowering stage. Observations on the flower made on the first day of flowering and at anthesis. Boll characters recorded at full maturity and before boll bursting. The traits like seed fuzz, fuzz color, and fiber color was recorded after ginning. Fiber quality traits like Length (2.5 % span length) (mm), fiber strength (g/tex), Fineness (Micronaire value), Uniformity (%) and Maturity (%) was measured in PRIMIER ART2 fully automated cotton testing instrument (ICC mode). The data on the state of expression of each trait was harmonized based on the finalized guidelines (Rathinavel *et al.*, 2005).

RESULTS AND DISCUSSION

The guidelines for the conduct of DUS testing in tetraploid cotton comprised 37 traits of which 22 are essential characteristics that are marked by an asterisk (*). These characteristics that shall be observed during every growing season on all varieties and shall always be included in the description of the variety, except when the state of expression of any of these characters is rendered impossible by a preceding phenological characteristic or by the environmental conditions of the testing region (Anonymous, 2008).

Distinctiveness implies that the variety should be distinguishable by at least one

essential characteristic from a variety which is a matter of common knowledge. Assessment of variability through the presence of different states of expression among released varieties of tetraploid cotton was assessed and the frequency distribution for various states of characteristics occurring in 54 varieties of tetraploid cotton has been presented based on the types of characteristic observed (Table 1, 2).

Grouping characters: Highly heritable characters are used for classification of varieties into different groups. The following characteristics are proposed to be used for grouping cotton varieties (Table 2).

- i) **Species** – Most of the varieties were *G. hirsutum* (52) and only two varieties namely Suvin and Sujatha were *G. barbadense*.
- ii) **Leaf: Shape (Characteristic 8)** - Majority of the varieties were palmate (52 varieties) and two *G. barbadense* varieties namely Suvin and Sujatha were semi-digitate.
- iii) **Flower: Petal colour (Characteristic 15)** - Cream was the predominant petal colour being recorded in 37 varieties followed by yellow colour in 15 varieties and two varieties recorded deep yellow.
- iv) **Flower: Pollen colour (Characteristic 19)** - For pollen colour, cream colour was observed in 30 varieties followed by yellow (22 varieties) and deep yellow (2 varieties).
- v) **Boll: Shape (longitudinal section) (Characteristic 23)** - Ovate boll shape was found in 47 varieties, round shape in 4 varieties and elliptic in 3 varieties.
- vi) **Fibre: Length (Characteristic 33)** - For fibre length, medium long (29), long (19), medium (4) and extra-long (2).

The results indicated that though variability exists for most of the traits, the majority of cotton cultivars released were of palmate leaf shape with cream coloured petal and pollen, ovate boll shape, and medium long fiber length.

Table 1. Frequency distribution of tetraploid cotton genotypes

S. No.	Characteristics	States
1	Hypocotyl : Pigmentation	Present (54)
2 (*)	Leaf: Colour	Light green (10), Green (44)
3	Leaf: Hairiness	Sparse (3), Medium (50), Dense (1)
4	Leaf: Appearance	Cup (45), Flat (9)
5	Leaf: Gossypol glands	Present (54)
6 (*)	Leaf: Nectaries	Present (54)
7	Leaf: Petiole pigmentation	Absent (30), Present (24)
8 (*)	Leaf: Shape	Palmate (52), Semi digitate (2)
9 (*)	Plant: Stem hairiness	Smooth (2), Sparse (6), Medium (44), Dense (2)
10	Plant: Stem pigmentation	Absent (2), Present (52)
11	Plant: Height (cm)	Semi dwarf (26), Medium tall (28)
12	Plant: Growth habit	Compact (30), Semi spreading (24)
13 (*)	Bract: Type	Normal (54)
14	Time of flowering (50%)	Medium (22), Late (32)
15 (*)	Flower: Petal colour	Cream (37), Yellow (15), Deep yellow (2)
16 (*)	Flower: Petal spot	Absent (52), Present (2)
17 (*)	Flower: Stigma	Embedded (50), Exerted (4)
18	Anther filament colouration	Absent (54)
19 (*)	Flower: Pollen colour	Cream (30), Yellow (22), Deep yellow (2)
20	Male sterility	Absent (54)
21	Boll: Bearing habit	Solitary (54)
22	Boll: Colour	Green (54)
23 (*)	Boll: Shape (longitudinal section)	Round (4), Ovate (47), Elliptic (3)
24 (*)	Boll: Surface	Smooth (52), Pitted (2)
25 (*)	Boll: Prominence of tip	Blunt (1), Pointed (53)
26 (*)	Boll: Opening	Semi open (2), Open (52)
27 (*)	Boll: Weight of seed cotton/boll (g)	Very small (15), Small (25), Medium (12), Large (2)
28 (*)	Seed: Fuzz	Naked (2), Sparse (2), Medium (44), Dense (6)
29 (*)	Seed: Fuzz colour	White (10), Grey (43), Brown (1)
30 (*)	Seed: Index (100 seed wt)	Small (8), Medium (24), Bold (17), Very bold (5)
31 (*)	Ginning (%)	Very low (3), Low (6), Medium (17), High (18), Very high (10)
32 (*)	Fibre: Colour	White (24), Cream (29), Brown (1)
33 (*)	Fibre: Length (mm)	Medium (4), Medium long (29), Long (19), Extra long (2)
34 (*)	Fibre: Strength (g/tex)	Weak (22), Medium (31), Very strong (1)
35	Fibre: Fineness	Coarse (1), Medium (20), Fine (19), Very fine (14)
36	Fibre: Uniformity (%)	Average (3), Good (22), Excellent (29)
37	Fibre: Maturity (%)	Average (1), Good (43), Very Good (10)

* Essential characteristics

Essential characteristics

In addition to the five grouping traits, 17 essential traits have been identified in the cotton DUS guidelines. These include 3 traits of leaf, one of stem, three of flower, four of boll, three of seed, and three of fiber traits. Ten cultivars exhibited a light green color and the remaining were green color (44). Leaf hairiness was medium state in 50, three in sparse and one variety in dense state. In stem hairiness, majority of varieties (44)

expressed medium hairiness followed by sparse in six, smooth and dense in each two varieties.

There was no variation in the state of expression of traits leaf nectaries (present), bract type (normal), anther filament coloration (absent), male sterility (absent), boll bearing habit (solitary), and boll color (green).

In the case of petal spots, the majority of the varieties possessed the absence of petal spots and only two varieties *i.e.*, Suvin and Sujatha (G.

Table 2. Grouping of tetraploid cotton varieties as per DUS characters

S. No.	Characters	Groups	No. of genotypes	Name of genotypes
1	Species	<i>G. hirsutum</i>	52	Abadhita, ACP 71, Anjali, Badnawar, Bikaneri Nerma, CSHH-198, Deviraj, F 846, F 1378, G.Cot. 10, G.Cot. 12, G.Cot. 16, G.Cot. 18, GSHV 112, Gujarat 67, JCC-1, JK 4, JLH 168, J 34, Kanchana, Khandwa2, Khandwa 3, Laxmi, LRA 5166, LH 900, L 604, MCU3, MCU 5, MCU5 VT, MCU 8, MCU9, MCU 10, MCU 11, MCU 12, MCU 13, Narasimha, NCH 11, NH 452, NH 545, NHH-44, PKV Rajat, Pratima, Reba B50, RMPBS 155, Sahana, SH 2379, Suman, Sumangala, Supriya, Surabhi, T7, VC 21
		<i>G. barbadense</i>	2	Suvin and Sujatha
2	Leaf: Shape	Palmate	52	Abadhita, ACP 71, Anjali, Badnawar, Bikaneri Nerma, CSHH-198, Deviraj, F 846, F 1378, G.Cot. 10, G.Cot. 12, G.Cot. 16, G.Cot. 18, GSHV 112, Gujarat 67, JCC-1, JK 4, JLH 168, J 34, Kanchana, Khandwa2, Khandwa 3, Laxmi, LRA 5166, LH 900, L 604, MCU3, MCU 5, MCU5 VT, MCU 8, MCU9, MCU 10, MCU 11, MCU 12, MCU 13, Narasimha, NCH 11, NH 452, NH 545, NHH-44, PKV Rajat, Pratima, Reba B50, RMPBS 155, Sahana, SH 2379, Suman, Sumangala, Supriya, Surabhi, T7, VC 21
		Semi digitate	2	Suvin and Sujatha
3	Flower: Petal colour	Cream	37	Abadhita, ACP 71, Anjali, CSHH-198, Deviraj, F 846, F 1378, G.Cot. 12, G.Cot. 16, Gujarat 67, JCC-1, JLH 168, J 34, LRA 5166, LH 900, L 604, MCU3, MCU 5, MCU5 VT, MCU 8, MCU9, MCU 10, MCU 11, MCU 12, MCU 13, Narasimha, NCH 11, Reba B50, RMPBS 155, Sahana, SH 2379, Suman, Sumangala, Supriya, Surabhi, T7, VC 21
		Yellow	15	Badnawar, Bikaneri Nerma, G.Cot. 10, G.Cot. 18, GSHV 112, JK 4, Kanchana, Khandwa2, Khandwa 3, Laxmi, NH 452, NH 545, NHH-44, PKV Rajat, Pratima
		Deep yellow	2	Suvin and Sujatha
4	Flower: Pollen colour	Cream	30	Abadhita, ACP 71, Anjali, Bikaneri Nerma, CSHH-198, Deviraj, F 846, F 1378, G.Cot. 10, G.Cot. 16, G.Cot. 18, JCC-1, J 34, Kanchana, Khandwa2, Khandwa 3, LH 900, L 604, MCU3, MCU 8, MCU9, MCU 11, Narasimha, NHH-44, PKV Rajat, Pratima, RMPBS 155, Sahana, SH 2379, VC 21
		Yellow	22	Badnawar, G.Cot. 12, GSHV 112, Gujarat 67, JK 4, JLH 168, Laxmi, LRA 5166, MCU 5, MCU5 VT, MCU 10, MCU 12, MCU 13, NCH 11, NH 452, NH 545, Reba B50, Suman, Sumangala, Supriya, Surabhi, T7
		Deep yellow	2	Suvin and Sujatha
5	Boll: Shape	Ovate	47	Abadhita, ACP 71, Anjali, Badnawar, Bikaneri Nerma, CSHH-198, F 846, F 1378, G.Cot. 10, G.Cot. 12, G.Cot. 16, G.Cot. 18, Gujarat 67, JK 4, JLH 168, J 34, Kanchana, Khandwa2, Khandwa 3, Laxmi, LRA 5166, LH 900, L 604, MCU3, MCU 5, MCU5 VT, MCU 8, MCU9, MCU 10, MCU 11, MCU 12, MCU 13, Narasimha, NCH 11, NH 452, NH 545, NHH-44, PKV Rajat, Pratima, Reba B50, RMPBS 155, Sahana, Suman, Sumangala, Surabhi, T7, VC 21

S. No.	Characters	Groups	No. of genotypes	Name of genotypes
		Round	4	GSHV 112, JCC-1, SH 2379, Supriya
		Elliptic	3	Deviraj, Suvin, Sujatha
6	Fibre: Length	Medium	4	JCC-1, NH 452, Reba B50, Suman
		Medium long	29	Abadhita, ACP 71, Bikaneri Nerma, CSHH-198, F 1378, G.Cot. 12, G.Cot. 18, GSHV 112, JK 4, JLH 168, J 34, Khandwa2, Khandwa 3, Laxmi, LRA 5166, LH 900, MCU3, MCU5 VT, MCU 8, MCU9, Narasimha, NH 545, NHH-44, PKV Rajat, Pratima, RMPBS 155, SH 2379, Sumangala, Surabhi
		Long	19	Anjali, Badnawar, Deviraj, F 846, G.Cot. 10, G.Cot. 16, Gujarat 67, Kanchana, L 604, MCU 10, MCU 11, MCU 12, MCU 13, NCH 11, Sahana, Sujatha, Supriya, T7, VC 21
		Extra long	2	MCU 5, Suvin

barbadense) having petal spots. Fifty varieties possessed embedded stigma and four varieties having exerted stigma.

Except for Suvin and Sujatha (pitted boll surface), all other varieties expressed smooth boll surface. All the varieties except Supriya produced bolls with pointed tip. In supriya it was blunt. In 52 varieties showed open type of boll opening, whereas Suvin and Sujatha were of semi-open.

The expression of boll weight was small, very small, medium, and large in 25, 15, 12, and 2 varieties, respectively. The fuzz state observed was medium in 44 varieties, dense in six and two varieties each in sparse and naked. In expression of seed fuzz color, Grey was the predominant (43 varieties) followed by white (10) and brown (1). Medium state of seed index was found in 24, bold in 17, small in 8, and very bold in 5 varieties. Ginning per cent was high in 18, medium in 17, very high in 10, low in 6 and very low in 3 varieties. Cream fiber color was predominant in 29 varieties followed by white in 24 and brown in one variety. The state of fiber strength observed was medium, weak, and very strong in 31, 22, and 1 variety, respectively.

Standard (Optional) characteristics

The optional characters were hypocotyl pigmentation, leaf appearance, leaf gossypol glands, plant stem pigmentation, plant height,

plant growth habit, days to 50 per cent flowering, anther filament coloration, male sterility, boll bearing habit, boll color, fiber fineness, and fiber uniformity. There was no polymorphism that exists among the traits like hypocotyl pigmentation, leaf gossypol glands, anther filament coloration, male sterility, boll bearing habit, and boll color.

The expression of trait leaf hairiness was medium (50), sparse (3), and dense (1). The appearance of leaf was cup in nature in 45 varieties, whereas flat in 9 varieties. The petiole pigmentation was observed present in 24 and absent in 30 varieties. Pigmented stem was noticed in most of the varieties except Suvin and Sujatha. The height of plant rated semi-dwarf in 26 varieties and the rest medium tall (28). Growth habit appeared as compact in 30 varieties and the remaining 24 was semi spreading. The time of flowering was found late in 32 varieties and in 22 this was medium.

Fine state of fiber fineness was noticed in 20 genotypes followed by medium (19), very fine (14), and coarse (1). Fiber uniformity recorded was Excellent in 29 varieties and the remain were good (22) and average (3)

Characterisation of fibre quality traits

The fiber quality traits evaluated as per DUS test guidelines expressed variation among the tetraploid cotton varieties. Fiber quality traits

are quantitative in nature and highly affected by the environment (Jiang *et al.*, 2000; Ulloa and Meredith, 2000). The evaluation of the 54 cotton varieties for fiber length, two varieties MCU5 and Suvin represent extra-long category, the remaining 9, 29, and 4 were long, medium long, and medium states, respectively. Fiber length is the normal length of a typical portion of the fibers of a cotton sample and it is directly related to yarn fineness, strength, and spinning efficiency. Longer fibers can be processed at greater efficiencies and produce finer and stronger yarns by allowing fibers to twist around each other more times, while shorter fibers require increased twisting during spinning, causing low-strength, poor-quality yarns (Chee *et al.*, 2005).

Fiber strength is also important because the inherent breaking strength of individual cotton fibers is considered to be the most important factor in determining the strength of the yarn spun from those fibers. In this study, variety Suvin exhibited very strong state and the rest 31, 22 were of medium and weak state. Fiber strength is related to the average length of the cellulose molecules deposited inside the cotton fiber, hence longer the cellulose chains, stronger the fiber. High fiber strength lines are desirable as they are directly correlated with yarn tenacity (Balakrishna *et al.*, 2016). Fiber fineness is another important factor in fiber quality because of its direct impact on processing performance and the quality of the end product. Among the 54 genotypes, 1, 20, 19 and 14 represented coarse (G. cot. 12), medium, fine and very fine states. Finer mature fibers can be spun into yarns with more fibers per cross-section, resulting in stronger and better quality yarns (Bradov and Davidonis, 2000). Cotton with micronaire value of 4.5 or greater are useful for non-woven roll goods as they have very few neps and fine fabrics are more prone to nep formation (Balakrishna *et al.*, 2016). In addition, fiber uniformity is also of tremendous value in the textile industry. It is highly correlated with the efficient spinning and

weaving processes which convert the fiber into fabrics. Fiber uniformity, recorded was excellent in 29, good in 22 and average in three varieties.

Among 54 varieties, seeds of six varieties showed dense fuzz, two varieties (Suvin and Sujatha) were naked and two varieties (PKV Rajat and LH 900) showed sparse, the remaining varieties seeds had medium fuzz. The fuzz colour was white in 10 varieties brown (JCC1) in one and in the rest grey color. Fiber color recorded was white in 24, cream in 29 and brown in one (JCC1) variety. The results revealed that the variations in fiber traits among the varieties were more of genetically controlled rather than environment.

CONCLUSION

The information generated on a wide range of variability and documented in the basic database served as a source of information for the selection of suitable reference varieties that are to be grown in the DUS with a new candidate variety for comparison and establishment of distinctiveness. Incorporation of states of essential traits which are not represented among the released varieties can form the basis for their distinctiveness in newly developed cultivars. In this study, 54 tetraploid varieties were characterised for 37 traits with different state of expression. The morphological data thus documented would also be useful for breeding varieties with distinct traits for fetching Intellectual property on plant varieties.

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