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Short communication

Novel multiple gynoecium genotype in sorghum (*Sorghum bicolor* L.)

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Abstract

A novel fodder sorghum (*Sorghum bicolor* L.) genotype (EC484238) with multiple gynoecium has been identified at ICAR-IGFRI, Jhansi for the first time in India. The floral microscopic study of this novel genotype revealed that female mega-gametophyte produced multiple gynoecium ranging from two to six, instead of one as in normal sorghum genotypes with bi-feathery stigma on each ovary. These multiple ovaries succeeded to produce viable twin or triplet seeds per spikelet per panicle. The twin and triple seed ratio ranged from 97-99% and 1-3%, respectively per panicle. This unique sorghum genotype might be a homeobox mutant which could shed light on the genetic regulation and control mechanisms governing the development of floral structure in sorghum as well as in other grasses of the Poaceae family. Multi-ovary sorghum has the obvious advantage of the increased number of seeds per panicle, thereby potentially increasing the seed yield. The novel genotype might be used in future fodder sorghum breeding programmes and will have more significance for the development of dual purpose fodder sorghum.

Keywords: Floral biology, Multiple gynoecium, Sorghum, Triple seed, Twin seed

Sorghum (*Sorghum bicolor* L., 2n=20, Family= Poaceae) is a multipurpose crop because of its diverse end-uses like feed, fodder, fuel and grain (Doggett, 1988; Suvarna *et al.,* 2020; Chand *et al.,* 2022). It ranked fifth among the top five cereal crops and is widely grown in the world, particularly in dry and hot climate (Goyal *et al.*, 2020; Laza *et al.,* 2022; Wang *et al.*, 2023). Sorghum inflorescence is a terminal panicle heads out from the flag-leaf sheath at heading time. Rachis is short or long with primary, secondary, and sometimes tertiary branches with spikelets in pairs and in groups of three at the ends of branches. Spikelet is sessile and bisexual or pedicelled/male/sterile with two florets. The sessile spikelet is long having glumes generally of equal length, two lodicules, three stamens, and one ovary superior with two long styles ending in bi-feathery stigmas (Stephens, 1934; Laza *et al.*, 2022). Sorghum is often-cross pollinated plant. The pollen grain remains viable for about 30 minutes after dehiscence or may be up to 2 to 4 hours depending upon the cultivar. After blooming, stigma remains functional for one day or up to a week (Schertz and Dalton, 1980). In sorghum, the average number of sessile spikelets per inflorescence is estimated approximately 2000 to 4000 and each spikelet has three anthers with an average of 5000 pollen grains in each (Ayyangar and Rao, 1936). The pollen grains germinate immediately after contact with the receptive stigma (Maunder and Sharp, 1963). The pollen tubes grow through the stigmatic style down the ovary. Fertilization between egg and sperm nuclei takes place within two hours and develops into an embryo (2n). Second fertilization occurs between polar nuclei and sperm and which develops into an endosperm (3n). However, there may be the chance of natural mutations in genes responsible for reproductive pathways regulation, resulting in alteration of the micro and megagametophytes cell differentiation and numbers. Consequently, such changes are reflected in spikelet shoot, seed shape, size and number (Stephens, 1936; Singhal *et al.*, 2022). A normal individual sessile spikelet consists of single gynoecia with three stamens. First time in India, we report here a novel sorghum genotype (EC 484238) characterized by multiple gynoecium ranging from two to six and produce twin and triplet seeded per sessile spikelet per panicle. The stamens

were three in normal condition. This communication discusses the characteristic features and their possible utilization in understanding the genetic regulation of floral biology in sorghum as well as in other members of Poaceae family.

Fodder sorghum genotypes (112) were grown in an augmented randomized block design at ICAR-IGFRI, Jhansi in the rainy season of 2022. During the germplasm characterization and evaluation for agro-morphology traits, a novel genotype having multiple gynoecium was observed, in contrast to the normal type having single gynoecia per sessile spikelet. The genotype was sown in four rows of 3m in length along with 30 cm x 15 cm of row to row and plant to plant spacing, respectively. All the panicles of each plant were covered with pollination bags from heading to grain-filling stage to ensure self-pollination and selfed seeds were harvested carefully. During post-rainy season of 2022, selfed seeds were again planted in four rows by following the same row length and row to row distance to confirm the trait stability. The novel genotype was also characterized for agro-morphological traits by following the DUS guidelines available in sorghum. The novel trait multiple gynoecium was confirmed through floral microscopic studies. Seed traits were also recorded on novel genotype with comparison to normal sorghum genotype.

During the flower initiation stage, four to six bi-feathery stigmas from a single sessile spikelet were observed. Further, floral microscopic study of this novel genotype revealed multiple gynoecium ranging from two to six and each bearing a well-developed style and bi-feathery stigmas. The number of gynoecia varied from two to six in a single spikelet (Fig 1a-e). After critical examination of each selfed-progenies, it was revealed that this unique trait had 100% expressivity and 100% penetrance and it was observed that there were no within line and between line variations which itself explained the stability, inheritance and trait were genetically controlled. During the flowering, differentiation of androecium and gynoecium occurred very early and was mostly complete and irreversible. However, some deviations from the normal differentiation were reported earlier in *Arabidopsis* (Goto and Meyerowitz, 1994), rice (Zhang *et al*., 2007), wheat (Peng, 2003) and sorghum (Karper, 1931). Joshi *et al*. (2015) reported two gynoecium in sorghum.

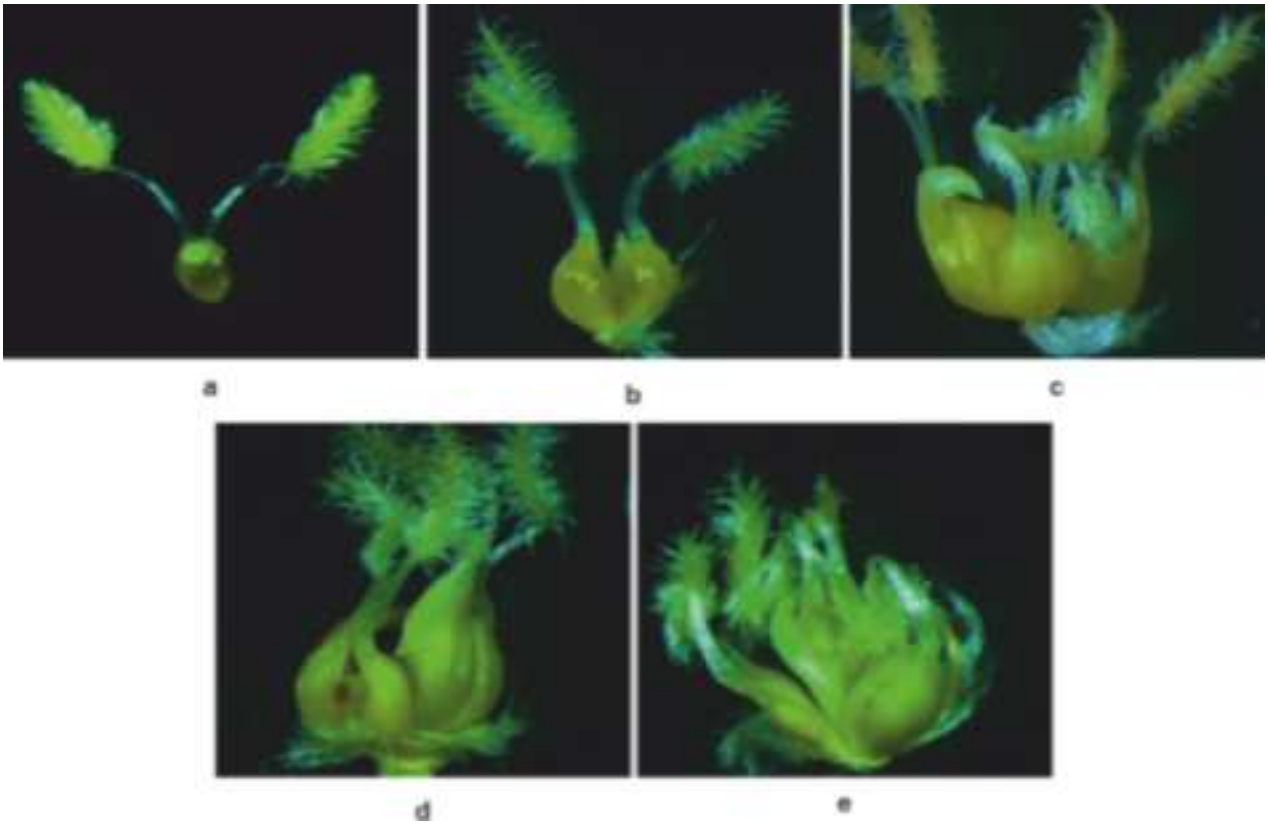

Fig 1 (a-e). Microscopic view of novel sorghum genotype (EC 484238) showing multiple gynoecium ranged from two to six in a single sessile spikelet

At the time of maturity, twin and triple seeds per spikelet were observed which might indicate to infer that among the multiple gynoecium (2 to 6), only two to three gynoecium were able to get fertilized and succeeded in developing twin or triple seeds per sessile spikelet (Fig 2a-b). The twin and triple seed ratio ranged from 97-99% and 1-3% respectively, per panicle. According to agro-morphological characterization it was observed that genotype was early flowering type (65 days), tall plant height (245.3 cm), long leaves (82.0 cm), broad leaf width (8.0 cm), medium stem girth (2.6 cm), flag leaf length (60.3 cm), flag leaf width (7.3 cm), long peduncle length (20.2 cm), and medium number of leaves (10) per plant (Table 1). These findings were in line with Chand *et al*., 2017; Goyal *et al*., 2020; Laza *et al*., 2022 and Bairwa *et al*., 2023.

For the seed related traits normal type and novel genotypes were taken for comparison in which five uniform panicles from each type genotypes were selected and tried to take nearly uniform panicles for comparison studies. Results showed that the 100-seed weight of normal genotype was 2.01 g, which was also observed earlier (Bairwa *et al*., 2023). In novel genotype, after separating twin and triple seeds from each other 100-seed weight was recorded 2.86 g, however, taking the twin and triple seeds as a whole (without separating from each other) it was recorded 5.4 g. Similarly, in case of mean values for number of seeds per panicle was counted 351 in normal type (Suvarna *et al.,* 2020). The number of seeds per panicle for novel genotype recorded 209.3 seeds when twin and triple seeds taken as whole seeds, without separating from each other while, after separating from each other and counted as individual seeds the mean value observed 418.7 seeds per panicle (Table 2). Results on seed traits revealed that twin and triple seeds in single spikelet did not affect the seed weight and shape in novel type sorghum genotype. Germination test was conducted for novel type genotype which showed that both the seeds were able to give rise to two separate coleoptiles suggesting that both seeds are viable and fertile. Nevertheless, the novel sorghum genotype reported in this study was unique as it produced multiple gynoecium ranging from two to six with twin and triple seeds per sessile spikelet per panicle which was not reported earlier in India. Even though a single spikelet was producing twin and triple seeds, individual seeds were of equal shape and size (Fig 3). The multiple gynoecium condition might be due to natural mutation in homeobox genes which were responsible for embryo development. This could be hypothesized that this novel sorghum genotype was a homeobox mutant.

The unique sorghum genotype with multiple gynoecium, twin and triple seeds could shed light on the genetic regulation and control mechanisms governing the development of floral structure in sorghum as well as in other grasses of the Poaceae family at molecular level. The novel genotype might be useful in future fodder sorghum breeding programmes and the resultant hybrid will have more significance for dual purpose type of fodder sorghum. To the best of our knowledge, it is the first report on a multiple gynoecium (2 to 6) along with twin and triple seeds per single sessile spikelet in India.

(a) **(b)**

Fig 2 (a-b). Expression of twin and triple seeds per panicle in the novel genotype sorghum genotype (EC 484238) during rainy and post-rainy season of the year 2022

Table 1. Agro-morphological characters recorded as per DUS guidelines in novel sorghum genotype (EC 484238)

Sr. No.	Parameters	Category	Mean value	Stage of observation
1	Seedling : Anthocyanin colouration of coleoptiles	Yellow green	-	Seedling 7-8 days after sowing
2	Leaf sheath: Anthocyanin colouration	Yellow green	-	5th leaf
3	Leaf : Midrib colour (5th fully developed leaf)	White	-	5th leaf
4	Time of panicle emergence	Early (56-65 days)	65	Panicle emergence (50% of the plants with 50% anthesis)
5	Plant : Natural height of plant upto base of flag leaf	Tall (226-300 cm)	245.3 cm	Panicle emergence
6	Flag leaf: Yellow colouration of midrib	Absent	-	Panicle emergence
7	Stigma: Anthocyanin colouration	Absent	-	Upper portion of the panicle at the end of flowering
8	Stigma: Yellow colouration	Absent	-	Flowering
9	Stigma: Length	Medium (1-2mm)	1.04 mm	Flowering
10	Flower with pedicel: Length of flower	Medium	0.8 mm	Flowering
11	Anther: Length	Short (<3mm)	3 mm	Flowering
12	Anther: Colour of dry anther	Orange red	-	End of flowering
13	Number of leaves per plant	Less (<15)	10	Physiological maturity
14	Stem girth (cm)	medium (2-3 cm)	2.6 cm	Physiological maturity
15	Number of tillers per plant	Medium (2-5)	2	Physiological maturity
16	Panicle: Length of branches (middle third of panicle)	Short (<5.1 cm)	4.8 cm	Physiological maturity
17	Panicle: Density at maturity (ear head compactness)	Compact	-	Physiological maturity
18	Panicle: Shape	Panicle broader in lower part Pyramidal	-	Physiological maturity
19	Neck of panicle : Visible length above sheath	Long (15.1-20 cm)	20.0 cm	Physiological maturity
20	Glume : Length	Very short	25% of grain covered	Physiological maturity
21	Grain: Threshability	Freely threshable	<11% unthreshed grain	Maturity
22	Caryopsis : Colour after threshing	White	RHS 155	After threshing
23	Grain : Shape (in dorsal view)	Circular	-	After threshing
24	Grain : Shape in profile view	Circular	-	After threshing
25	Grain: Luster	Non-lustrous	-	After threshing

Table 2. Panicle traits and seed count per panicle in normal and novel type sorghum genotype (EC 484238)

Traits	Normal (PC-6)	EC 484238
Panicle length (cm)	13.1	13.4
Panicle width (cm)	4.9	3.7
Panicle weight (g)	12.2	11.2
Seeds per panicle	351	209.3[a]
	-	418.7[b]
100 seed weight (g)	2.01	5.4[a]
	-	2.86[b]

[a] (without separating twin and triple seed from each other); [b] (after separating twin and triple seeds from each other)

Fig 3. Studies conducted for comparison between normal and novel sorghum genotype (EC484238); A. Panicle: a) normal and b) novel genotype; B. Seeds: a) twin and triple seeds after separating from each other b) normal type seeds; C. Germination test of novel type sorghum genotype

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