Insight into floral biology and ancillary characteristics of underutilized legume-Bambara groundnut [Vigna subterranea (L.)Verdc.]

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ABSTRACT
The present study floral biology and ancillary characteristics of Bambara groundnut used SB-42, Uniswa Red, S-165-A, S-193 and nine isolated gamma irradiated stabilized mutants of SB-42 for this investigation. Mutant 11 produced significantly maximum number of pods per plant (46.05 g/plant) and seed yield per plant (28.96 g/plant), compared to control SB-42 (40.85 g/plant, 20.54 g/plant) respectively. Results obtained from flower biology studies were, Inflorescence- Simple Racemes, Flower- Bracteates, Zygomatic, Hermaphrodite, Pedicellate, hypogynous, Calyx- sepal 5, gamosepalous, valvate aestivation, Corolla- petals 5, Polypetalous, Papilionaceous with a standard (veilum/banner) petal, 2 wing petals(Alae) and 2 keel petals (Carinae), Androecium- stamens 10, Didelphous condition (9+1), Gynoeceum- Ovary superior, Floral formula- Br Brl Q K C A (1) and (K₃C₅₂₅₅₂₄₄₄₃G (1). Key words: Ancillary traits, Bambara groundnut, Flower, Mutant, Seed yield.

INTRODUCTION
Bambara groundnut is an African legume cultivated mainly by subsistence farmers under the traditional low input agricultural system. It serves as an important source of protein (19 % -28 %) in the diets of a large percentage of the population in Africa, particularly to poorer families who cannot afford expensive animal protein (Bamishaiye et al., 2011). Many people might be confuse that Bambara groundnut is a cultivar of groundnut, but it is an underutilized legume crop known for high protein content (Chandra et al., 2017 b). It is neglected and underutilized crop which play prominent roles in sustaining the impoverished rural population by supplementing a combination of low sodium and high potassium rich food indicated a value of protective sodium to potassium ratio (less than one) that is 0.04 significantly decreases risk of cardiovascular disease (Chandra et al., 2017 a). The crop has originated in the Sahelian region of present day West Africa, from the Bambara tribe near Timbuktu who now live mainly in Central Mali, (hence it’s name Bambara Groundnut). Bambara groundnut has been introduced into India with the introduction of ten entries namely, S16-5A, S19-3, SB-42, Gabc, DODR-TZ, DODC-TZ, DIPC, Uniswa Red, AS-17, AHM-753 from the University of Nottingham, U.K. through National Bureau Of Plant Genetic Resourses, New Delhi based on the indent of National Research Centre for Groundnut, Junagadh, Gujarat, India during the year 2002. The genotypes have since been tested at different locations in the states of Andhra Pradesh, Gujarat, Karnataka and even at BARC, Mumbai.

Male fertility depends on the rate of pollen production and viability and, it is strongly influenced by environment (Khatun and Flower, 1994). Pod and seed development take place approximately 30 to 40 days after fertilisation. The fruit of Bambara groundnut develops on or below the soil surface. The pod develops first, this takes up to 30 days after fertilisation. The seed develops during a further period of 10 days. Bambara groundnut is probably the most drought resistant grain legume and may be found surviving successfully where annual rainfall is below 500 mm and optimum between 900–1000 mm per year. The crop has days to maturity periods of 4-5 months (Bamishaiye et al., 2011). Given the rapid rate at which the world’s population is currently increasing in relation to agricultural production, the goal of research must be to improve the productivity not only of our main crops, but also certain neglected crops. Research priorities needs to be reorganized in order to attend adequately to the development of our local underutilized crops, like Bambara groundnut. In this regard it becomes pertinent to explore this crop. Varietal improvement of Bambara groundnut has been largely difficult due to the autogamous and cleistogamous flower nature of the crop (Chandra et al., 2016). Hence the present study was undertaken to study the flower biology and variability for ancillary characters.

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MATERIAL AND METHODS
Experimental location and station: The present study was carried out at K-block, University of Agriculture Sciences, Bengaluru during Kharif season of 2014-15. Geographically, the place is located at 12°58' latitude north and 77°35' longitude east. The center is at an altitude of 930 meters above sea level. The annual rainfall ranges from 679.1 mm to 888.9 mm.

Experimental material and methodology of the mutagenesis: Bambara groundnut varieties SB-42, Uniswa Red, S-165-A, S-193 and nine isolated gamma irradiated stabilized mutants of SB-42 were used for present investigation. The seeds were obtained from National Research Centre on Groundnut Junagadh through National Bureau of Plant Genetic Resources. SB-42 variety is an adapted variety to the semi-arid tracts of Karnataka hence dry seeds were packed in separate poly bags at the rate of 100 seed per packet for each treatment and sent for irradiation treatment to BARC, Trombay, Mumbai. The gamma rays from a 60Co source were used as mutagen.

Optimum dose of Ld50 was found to be 200GY. The variety SB-42 treated with gamma rays grown in 2010, M1 generation were raised and from M2 generation selection was carried out based on economic characters (Vijaya Kumara, 2011). These selected mutants advanced in plant to progeny rows till M6 generation (Veeraghanti, 2012). For the present investigation 9 stabilized mutants were selected from M6 population based on yield more than SB-42 and four varieties which were procured from Junagadh were used. Seeds were sown in two replication during kharif season on 14 August 2014 under rainfed condition. A spacing of 30 cm between rows and 15 cm within a row was followed at K block, GKVK, Bengaluru. N: P: K fertilizers dose of 25:75:38 kg per hectare applied. Suitable management practices were undertaken during the period of the crop growth. Plant and seeds of Bambara groundnut is shown in Figure 1 and 4 respectively.
Floral diagnosis done during anthesis phase on five flowers, followed by dissection. Information on genetic variability is the source for different characters is a prerequisite before initiating breeding programme aimed at improving yield and other characters under consideration. Expression of characters like yield and yield components is governed by a number of genes. The variability for these characters can be either on positive or the negative side depending on the gene affected by the mutation and the role played by the concerned gene in the expression of character. Study of the extent of variability for traits is important in ascertaining whether mutation breeding can be resorted for improvement of the character. In the present study, effect of variation was studied in the form of the mean, range, coefficient of variance and critical difference value for thirteen characters viz., days to germination, germination %, plant height at flower bud initiation (cm), no of branches at flower bud initiation, no. of leaves at flower bud initiation, no. of pods per plant(g), plant dry weight(g), shelling %, pod yield per plant (g), seed yield per plant(g). The success in crop improvement of Bambara groundnut depends on the ability of a breeder to define and assemble the required genetic variability and to select for yield indirectly through yield associated characters.

RESULTS AND DISCUSSION

The present study deals with four varieties and gamma rays induced mutants of SB-42 in Bambara groundnut with regard to flower biology and ancillary characteristics. The results of the present study have been described under appropriate headings and subheading.

Studies on flower biology: Flowers are the reproductive organs of a plant. Sexual reproduction in plants is enabled by flowering and is a sign of plant survival. Flower biology studies were conducted during anthesis period and following results have been obtained. Bambara groundnut flower and its description with different part of flower and flower diagram have been presented in Figure 2 and 3 respectively.

Floral formula- Br Brl $Q^{+} K_{(2)} C_{1+2} A_{9+1} G_{(1)}$

Bract is a modified or specialized leaf, especially one associated with a reproductive structure such as a flower, inflorescence axis or cone scale. Bracts are often different from foliage leaves. They are smaller look different from the parts of the flower, such as the petals and/or sepals. The state of having bracts is referred to as bracteates and they also have bracteole or bracteolate. Bambara groundnut flower is papilionaceous flowers (from Latin: papilion, a butterfly) are flowers with the characteristic irregular and butterfly-like corolla, which is found in many, though not all, plants of the species- Faboideae subfamily of legumes. The flowers have a bilateral symmetry with the corolla consisting of five petals. A single, large, upper petal is known as the banner (also vexillum or standard petal). It has indeterminate type of inflorescence bearing pedicellate flowers. Indeterminate inflorescence like raceme, the oldest flowers are borne towards the base and new flowers are produced as the shoot grows, with no predetermined growth limit. Flowers having short floral stalks called pedicels along its axis. In botany, axis means a shoot, in this case one bearing the flowers. It has zygomorphic flowers means it can be divided by only a single plane into two mirror-image halves. Aestivation or estivation refers to the positional arrangement of the parts of a flower within a flower bud before it has opened. In this crop petals have vexillary or descending imbricate type of aestivation in which the anterior petal is innermost and posterior petal is outermost & largest. The stamens have a diadelphous morphology and a superior ovary is an ovary attached to the receptacle above the attachment of other floral parts.

Studies on flower biology of Bambara groundnut inferred that, the flowering habit of the crop was indeterminate; hence flowering is likely to result in low yield since all flowers produced before harvesting will not produce mature seeds. This result is in confirmation with Doku and Karikari (1970). The present study also revealed that the crop possessing typical papilionaceous flower with one standard, two wing and two keel. Since the flower buds are normally located in the axil of petiole, the emasculated or hybridized flowers are prone to disease and damage associated with rain and watering.

Leaves are pinnately trifoliate with erect petiole, thickened at the base. The flowers are borne on hairy peduncle which arises from nodes of the stem. This result is in favour of Doku and Karikari (1971). Brink (1997) indicated that in Bambara groundnut, greatest difference in the time from 50 % flowering effects on flowering and podding, it seems more appropriate to separate two phases completely, and not include time to flowering in the podding analysis. Kumaga et al. (2002) had stated that rainfall and temperature appeared to be the two most important climatic factors that influence vegetative growth, flowering and yield of Bambara groundnut in Ghana. Bambara groundnut produced greater number of leaves in the major season than in the minor season and they attributed it to increased physiological activity. Mbagwu et al., (2006 a) stated that V. subterranea is having actinomorphic floral symmetry however author observed it is zygomorphic.
Flowers are presented as papilionaceous racemes. The bulbous tip creates a tunnel through which the fertilized flower, attached just behind the tip, is drawn into the soil. It is a monoecious, self-pollinating annual with tremendous potentials as a food crop and a soil ameliorating agent. The crop has a perfect flower with the stamen and pistil borne in same flower. This structure offers protection to the male and female parts of the flower and also discourages outcrossing to a large degree. Male fertility depends on the rate of pollen production and viability and, it is strongly influenced by environment. The flowers are borne on hairy peduncles, which arise from the nodes of the stems. Usually, two flowers are attached to the peduncle by pedicels. A good knowledge of the flower structure is essential for breeding the crop. The flower has a pair of hairy epicalyces. The calyx consists of five hairy sepal (four on the upper side and one on the lower side).

The epicalyx and calyx completely enclose the corolla in the early budding stage. The epicalyx drops off during the course of entry of the fertilized flower into the soil, but the calyx persists on the developing pod. The standard encloses the wing and keel until the flower opens. When the standard petal opens, it is bent over to about half its length. The stamens are diadelphous, nine with partly fused filaments, and one isolated vexillary stamen. Upon pollination and fertilization, the peduncle elongates to bring the ovaries to or just below ground level.

**Variability for ancillary characteristics of different varieties and mutants of Bambara groundnut:** Information on genetic variability is the source for different characters is a prerequisite before initiating breeding programme aimed at improving yield and other characters under consideration. Expression of characters like yield and yield components is governed by a number of genes. The variability for these characters can be either on positive or the negative side depending on the gene affected by the mutation and the role played by the concerned gene in the expression of character. Study of the extent of variability for traits is important in asserting whether mutation breeding can be resorted for improvement of the character. The success in crop improvement of Bambara groundnut depends on the ability of a breeder to define and assemble the required genetic variability and to select for yield indirectly through yield associated characters.

Considering the number of days from sowing to seedling emergence varied from 11.80 to 16.10 days. Six genotypes (S-165 A, S-193, mutant 2, 15, 19 and 24) took longer time, more than 14 days to emerge (Table 1). This may be attributed to the thickness of the seed coats and under conditions of low rainfall, early emergence would be advantageous. Rapid emergence reduces the period over which seedlings are susceptible to stress and the quicker the roots develop, the more likely the seedling is able to withstand drought. Early germination was observed for SB-42 variety (11.80 days) followed by its mutant 11(12.35 days) (Table 1). A decrease in germination % is taken as marker for genetic and physiological damage caused by a mutagen. Under study the mutagen has shown adverse effect on most of the mutants except mutant 11 and 19 for germination %. Decrease in the germination % due to the effect of mutagen was also reported by Sharma et al. (2005) in black gram, and Singh and Rao (2007) in green gram.

**Table 1:** Mean value of ancillary characteristics of different varieties and mutants of Bambara groundnut.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>DTG</th>
<th>GP</th>
<th>PH</th>
<th>NOB</th>
<th>NOL</th>
<th>NOP</th>
<th>PDY</th>
<th>SP</th>
<th>PY</th>
<th>SY</th>
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<td>30.95</td>
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<td>85.11</td>
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<td>Minimum</td>
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<td>53.67</td>
<td>22.15</td>
<td>17.30</td>
<td>31.75</td>
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<td>12.35</td>
<td>71.43</td>
<td>16.28</td>
<td>11.73</td>
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<tr>
<td>Maximum</td>
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<td>88.67</td>
<td>28.96</td>
<td>27.25</td>
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<td>46.05</td>
<td>19.56</td>
<td>85.11</td>
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<td>73.08</td>
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<td>1.48</td>
<td>9.03</td>
<td>2.28</td>
<td>4.37</td>
<td>-</td>
<td>8.07</td>
<td>-</td>
<td>7.99</td>
<td>6.62</td>
<td>4.55</td>
</tr>
</tbody>
</table>

**F test** | 5.45** | 12.90** | 7.91** | 4.50** | NS   | 8.93** | NS   | 3.74* | 5.13** | 9.33**

**Significance at 1 % level, * significance at 5 % level, DTG- days to germination, GP- germination %, PH- plant height at flower bud initiation (cm), NOB- No of branches at flower bud initiation, NOL- No. of leaves at flower bud initiation, NOP- No. of pods per plant(g), PDY- Plant dry weight(g), SP- shelling %, PY- Pod yield per plant (g), SY- Seed yield per plant(g).**
Plant height at flower bud initiation considered to be an indicator of the appropriate period of emasculation at mean height of 25.88 (Table 1). It was observed that there is a reduction in mean plant height of mutant 11 and 19 compared to its control (variety SB-42), whereas for remaining mutants there was an increase in plant height. The increased variability offers a scope for selection and improving the character in a desired direction. These results are in confirmation with the findings of Ganguli and Bhandari (1980) in green gram. Among four varieties, minimum plant height observed was for SB-42 followed by S-165 A, Uniswa Red and S-193. This may be due to the varietal characteristics.

Mutant 11 produced significantly maximum number of pods per plant (46.05 g/plant) compared to control SB-42 (40.85 g/plant) (Table 1). This showed that this genotype partitioned more of the dry matter into production of more pods per plant; however it was also observed that the mean number of pods per plant decreased in mutants when compared to control except mutant 11. Wider range was observed compared to the control. Results of Khan (1985) and Mohd and Khan (2006) indicated mean number of pods per plant increased in the mutant population of mung bean compared to control.

Shelling percentage is a reflection of pod filling efficiency and high shelling percentage values indicate effective pod filling. Mutant 11 recorded significantly the highest shelling percentage of 85.11 % (Table 1). This may be due to efficient partitioning of assimilates into the seed rather than the husk. Variability for pod yield per plant in Bambara groundnut genotypes indicated the mean pod yield per plant decreased in all mutants except mutant 11 and 15 when compared to control. This result was in confirmation with Ganguli and Bhandari (1980) in mung bean.

Mutant 11 was highly and significantly more productive than remaining genotypes of Bambara groundnut and thus again showing the inherent ability of partitioning most of the dry matter produced into the grain. High economic yields are predetermined by dry matter production and partitioning into various sinks of which the grain is the most important. Therefore, any attempt to maximize yield and cultivar assessment for higher yield are considered successful if subsequent growth characteristics support dry matter partitioning to seed. Mutant 11 also produced maximum numbers of pod and highest seed yield. A higher seed yield is again attributed to the higher number of pod produced.

CONCLUSION

Bambara groundnut is a popular crop because of its resistance to drought and the ability to produce a reasonable crop when grown on poor soils. It is the third most important crop after groundnuts and cowpeas but it has low status as it is seen as a snack or food supplement but not a lucrative cash crop. Reports on critical genetics and the breeding of Bambara groundnut are limited. To date, the full genetic diversity of the crop remains largely unexploited. Until recently Bambara groundnut never received any appreciable research effort, especially for its genetic improvement. Until then, only selection breeding was practised in which existing landraces were evaluated and their seeds multiplied. However, no new combinations resulting from hybridization had ever been produced. Hence this work will give a basic platform to understand its importance and available variability including its flower biology information, which can be utilized for further breeding strategy.

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