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Assessing the Pattern of Seed Development and Maturation in Yard Long Bean [*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt]

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ABSTRACT

Background: Seed maturation is genetically controlled process involves a sequence of morphological and physiological changes extending from fertilization to total independence from the mother plant. Yard long bean (*Vigna unguiculata* subsp. *sesquipedalis* (L) verdcourt) is an important leguminous vegetable crop which meets greater demand of the vegetable especially in South India and some parts of North India. However, information on optimum harvest time of yard long bean seeds are still limited. Hence, this study was carried out to determine the physiological maturity of yard long bean to obtain good quality of seeds for better planting value.

Methods: The laboratory experiment was carried out at Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore, India to determine the appropriate time of harvesting yard long bean cv. Arka Mangala seeds. The crop was raised as bulk in the field during *kharif* season of 2019 and the pods were harvested at three days interval from 3 days after anthesis (DAA) to 30 DAA and subjected for determinations of pod and seed characteristics.

Result: The results revealed that pod length, pod fresh weight and pod dry weight increased rapidly during 3 DAA to 12 DAA and showed maximum pod length (67.7 cm), pod fresh weight (28.56 g/pod) at 12 DAA. The seeds attained physiological maturity at about 24 DAA with 22 per cent moisture content concurred with maximum dry weight (18.80 g/100 seeds), protein content (18.6%) and maximum physiological parameters *viz.,* speed of germination (7.1), germination per cent (94%), root length (23.5 cm), shoot length (20.5 cm), dry matter production (0.949 g/10 seedlings), vigour index I (4136) and vigour index II (89).

Key words: Germination %, Good quality seeds, Physiological maturity, Yard long bean.

INTRODUCTION

Yard long bean [*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt] also called as long podded cowpea, asparagus bean, Chinese long bean, bodi *etc.* is a distinct form of cowpea grown in many parts of India. It is a rich and inexpensive source of vegetable protein. It enriches fertility of the soil by fixing atmospheric nitrogen. It has become an essential component of sustainable agriculture in marginal lands of the tropics because of its quick growth habit. It is highly nutritive vegetable containing a good amount of digestible protein both in pods (23.5-26.3%) and in leaves. In addition to that, they are good source of micronutrients containing iron (102.7-120 mg), zinc (32.6-36.7 mg), manganese (2.9-3.3 mg) and cobalt (0.3-0.6 mg) (Ano and Ubochi, 2008). A serving of 100 g of yard long bean contains 50 calories, 9.0 g of total carbohydrates, 3.0 g of proteins, 0.2 g total fat and 0.8 g of minerals (Anonymous, 2013a).

The flowers in the plant or in the same inflorescence generally does not pollinated at the same time, hence the uniformity of seed maturation is never achieved. According to Delouche (1971), seed maturation is a series of morphological, physical, physiological and biochemical changes that occur from ovule fertilization to the time when seeds become physiologically independent of the parent plant. The metabolic activities of seeds can be divided into four phases. Phase I and II comprise cell division and expansion. Reserve accumulation occurs in Phase III as seed dry mass increases. At the end of this phase, seed moisture loss is intensified (Phase IV).

Until the seed reaches complete maturity, full viability and germination of seed cannot be attained. Obtaining high quality seeds will depend on the ideal harvest moment and such moment should correspond to maximum seed quality. However, information on ideal harvest time and physiological maturity of yard long bean seeds are still scarce, especially for the variety Arka Mangala. Therefore, this study was aimed to assess the pattern of seed development and maturation in yard long bean by monitoring changes in physical, physiological and biochemical characteristics of seeds.

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MATERIALS AND METHODS

The experiment was conducted at Department of Seed Science and Technology, Tamil Nadu Agricultural University (TNAU), Coimbatore, India during *kharif* season of 2019. Yard long bean seeds obtained from Indian Institute of Horticultural Research (ICAR - IIHR), Bangalore, Karnataka, India formed the base material for this study. The crop was raised as bulk at orchard, TNAU (11°00′29″N, 76° 55′49″E) with recommended package of practices (Anonymous, 2013b) and about 200-250 individual flowers were tagged daily at the time of anthesis. Pods were harvested from 3 day after anthesis (DAA) until 30 DAA with three days intervals. The laboratory experiment was conducted in a completely randomized design (CRD) with ten treatments (DAA) and four replications to each treatment.

At each stages (DAA), ten pods each in four replications were taken to measure the pod length (cm), pod width (mm) and pod fresh weight (g/pod). After determining the fresh weight, the pods were longitudinally bisected and then dried in a hot air oven (80°C), until the pod reached constant weight. At the end of the prescribed period, the container was placed in a desiccator to cool for 30-40 minutes and the dry weight of pods were weighed and expressed in g/pod. Moisture content of pod was calculated by deducting the weight of dried pod from the fresh weight and expressed as percentage to the weight of fresh pods. The growth rate (cm or mm/day) of pod and seed was calculated by using the formula given by Briggs *et al.* (1920) per day basis. Pod chlorophyll content was estimated by acetone method (Arnon, 1949).

At every stages (DAA), twenty five seeds each in four replications were taken to measure seed length and seed breadth by using stereo zoom microscope. Immediately after harvesting of pods, the seeds were manually extracted and the parameters *viz.,* seed fresh weight (g/100 seeds), seed dry weight (g/100 seeds) and moisture content % (ISTA, 2015) were estimated.

$$\text{Moisture content (\%)} = \frac{\text{Differences in fresh and dry weight}}{\text{Fresh weight of the sample}} \times 100$$

The speed of germination was determined by adopting Maguire (1962) method. The germination test was conducted with four replicates of hundred seeds each using sand media and the set-up was placed in a germination room maintained at 25±2°C and 95±3% RH, illuminated with fluorescent light. After the test period of eight days, the emerged normal seedlings in each replications were counted and the mean was expressed in percentage (ISTA 2015). From each replications, ten normal seedlings were used for measuring the parameters *viz.,* root length (cm), shoot length (cm) and dry matter production (g/10 seedlings). Vigour index (VI) was computed by Abdul-Baki and Anderson (1973) method and the values was expressed as whole number. Protein content (%) of seed was determined by Ali-Khan and Youngs method (1973). For determining electrical conductivity (EC), four replicates of twenty five seeds from each stages (DAA) were soaked in 50 ml of distilled water for 24 hours and it was expressed as μSm^{-1} (Presley, 1958).

Vigour index I = Germination (%) × Mean seedling length (cm)
Vigour index II= Germination (%) × Dry matter production (g)

Statistical analysis

Statistical analysis was done as per the procedure described by Gomez and Gomez (1984). The data taken as per cent were transformed to the respective angular (arcsine value) before subjecting them to statistical analysis. The critical difference (CD) was worked at 5 per cent (P = 0.05) level and wherever 'F' value is non-significant it is denoted by "NS".

RESULTS AND DISCUSSION

Changes in pod characteristics during development and maturation

Highly significant changes were found for all the evaluated pod at each stages of development and maturation. Embryogenesis phases involved intense cellular division and expansion of pod up to 12 DAA. These leads to progressive increase in pod length and it became maximum at 12 DAA. At this stage, the maximum pod length (67.7 cm) was attained (Table 1) and after this stage there was no significant increase in pod length and its growth was ceased. Similar finding of increasing trend in pod length was reported in cluster bean by Renugadevi *et al.* (2006). Data from Table 1 shows that pod width increased rapidly up to 12 DAA due to cell division and extension of pod and the maximum pod width (13.5 cm) was observed at 12 DAA. The rapid growth rate of pod length after fertilization to 12 DAA might be due to more uptake of water, nutrients and also accumulation of photosynthates from source to sink which was positively correlated with increased fresh weight of pod. Similar finding was reported by Deshmukh *et al.* (2011) in developing cowpea pod.

Table 1 shows that three distinct stages were observed during pod development. During the first stage of pod development happened at 3 DAA to 12 DAA, there was rapid accumulation of dry matter coincided with moisture content of 72.6% (12 DAA) for pods. During the second stage of pod development occurred at 12 to 24 DAA, there was rapid drop in fresh weight of pod and the moisture content dropped to about 27.9% (24 DAA). The third stage was categorized by a gradual loss of moisture, with pod moisture content of 21.1% at 30 DAA. Similarly, three stages of pod development was reported for cowpea (Palanisamy *et al*., 1986).

Due to high water content, the fresh weight of pod was high and as maturation progresses the fresh weight decreased and the dry weight increased. The fresh weight of the pod reached the maximum of 28.56 g/pod at 12 DAA which was supported by increase in morphological structure of pod (Table 1). Similar result was reported by Fakir *et al.* (2013) in *Dipogon lignosus* during the maturation period. Table 1 shows that pod moisture content decreased from 89.5 to 21.1 per cent during the maturation period (3 DAA to 30 DAA) and that this could be due to replacement of osmotic

Table 1: Effect of days after anthesis (DAA) on pod characteristics of yard long bean cv. Arka Mangala.

Days after anthesis (DAA)	Pod length (cm)	Pod width (mm)	Pod growth rate		Fresh weight of pod (g/pod)	Dry weight of pod (g/pod)	Pod moisture content (%)	Chlorophyll content (mg/g of pod)		
			Length (cm/day)	Width (mm/day)				Chlorophyll a	Chlorophyll b	Total chlorophyll
3	8.1	3.0	-	-	0.19	0.02	89.5 (71.09)	0.841	0.236	1.077
6	30.3	4.8	.40	0.60	5.56	1.03	81.5 (64.53)	0.585	0.197	0.782
9	49.5	7.8	6.40	1.00	15.23	3.47	77.2 (61.48)	0.203	0.053	0.257
12	67.7	13.5	6.07	1.90	28.56	7.82	72.6 (58.44)	0.153	0.039	0.196
15	67.6	13.4	-0.03	-0.03	25.89	8.25	68.3 (55.73)	0.046	0.015	0.061
18	67.6	13.0	0.00	-0.13	17.12	8.21	51.8 (46.03)	0.008	0.002	0.010
21	67.8	13.2	0.07	0.07	12.34	8.24	33.2 (35.18)	-	-	-
24	67.8	13.1	0.00	-0.03	11.45	8.26	27.9 (31.88)	-	-	-
27	67.9	13.1	0.03	0.00	10.78	8.29	23.1 (28.73)	-	-	-
30	67.8	13.1	-0.03	0.00	10.56	8.23	21.1 (27.35)	-	-	-
Mean	56.2	10.8	2.21	0.37	13.77	6.18	54.6 (48.04)	0.306	0.090	0.397
SEd	0.725	0.166	0.050	0.012	0.213	0.095	0.634	0.008	0.002	0.003
CD (P=0.05)	1.481	0.338	0.103	0.024	0.435	0.194	1.294	0.017	0.004	0.006

Figures in the parenthesis indicate arcsine values.

material by starch and other large molecules with low hydration capacity. Similar finding of decrease in moisture content of pod was noted in lablab by Manohar (1970).

Table 1 shows that pod chlorophyll content analyzed at different DAA followed a decreasing trend with increasing maturity of pod. Chlorophyll a, b and total chlorophyll content decreased during the pod development period and completely ceases at 18 DAA. This was due to utilization of chlorophyll pigments for pod growth and development which was positively correlated with increases in pod growth. Manimurugan (2016) also reported the similar finding of decreasing chlorophyll content in carrot during maturation period.

Changes in pod length and fresh weight are the useful indicators to determine the appropriate time of harvesting yard long bean pods. Timely harvest is important to avoid problems of fibrous fresh pods. For high yield and market quality of fresh pods, yard long bean must be harvested at 12 DAA before seeds mature. Pods must be harvested at maximum pod length (67.7 cm) and pod fresh weight (28.56 g/pod) but before seeds become visible as bumps on the outside of the pod (Table 1). Similar finding was also reported by Das and Fakir (2014) for harvesting *Lablab purpureus*.

Physical changes of seed during development and maturation

Harrington (1972) stated that physiological maturity is the stage at which the seed reaches its maximum dry weight and nutrient flow into the seed from mother plant is ceased by the formation of abscission layer and causes breakage of vascular connection (Eastin *et al*., 1973).

During development and maturation of seeds, four phases was observed. At phase I and II (3 DAA to 15 DAA), there was significant increases in seed size due to rapid cellular division and expansion. At 15 DAA, the seed length and width was 12.92 mm and 6.32 mm respectively and after that there was no significant increase in seed size (Table 2). Maximum fresh weight was attained at 15 DAA and decreased thereafter due to reduced cell division and depletion of moisture content from seed. Similar results were observed by Krishnakumary (2012) in vegetable cowpea varieties due to desiccation drying. Seed moisture content during this phase decreased slowly because water is the vehicle for transferring nutrients from parent to developing seeds.

At phase III (15 DAA to 24 DAA), maximum dry weight (18.80 g/100 seed) of seed was attained at 24 DAA (Table 2) and after this stage there was no increase in dry weight of seed due to disintegration between source and sink. Between 15 DAA and 24 DAA, there was rapid loss of moisture from the seeds due to reduced cell division and replacement of moisture by reserve materials. At phase IV (24 DAA to 30 DAA), the moisture content decreased gradually and hygroscopic equilibrium was attained at 27 DAA. During this phase, there is no significant increase in dry weight of seed due to separation of seeds from the pod. From this point onwards, moisture content changes was associated with variations in relative humidity. This four phases of seed development and maturation was in

Table 2: Effect of days after anthesis (DAA) on seed physical and biochemical characteristics of yard long bean cv. Arka Mangala.

Days after anthesis (DAA)	Seed length (mm)	Seed breadth (mm)	Seed growth rate		Fresh weight of seed (g/100 seed)	Dry weight of seed (g/100 seed)	Seed moisture content (%)	Seed protein (%)	EC (μSm^{-1})
			Length (mm/day)	Width (mm/day)					
3	1.68	0.72	-	-	-	-	-	-	-
6	5.62	1.65	1.31	0.31	2.30	0.45	80.4 (63.72)	4.3 (11.97)	95.3
9	9.58	3.77	1.32	0.71	9.67	1.99	79.4 (63.01)	7.2 (15.56)	167.3
12	11.47	5.32	0.63	0.52	24.45	7.38	69.8 (56.66)	10.4 (18.81)	478.3
15	12.92	6.32	0.48	0.33	32.45	14.56	55.1 (47.93)	13.2 (21.30)	689.5
18	12.91	6.30	0.00	-0.01	28.89	16.87	41.6 (40.16)	15.4 (23.11)	843.7
21	11.65	5.86	-0.42	-0.15	24.99	17.47	30.1 (33.27)	17.2 (24.50)	758.9
24	11.64	5.85	0.00	0.00	24.09	18.80	22.0 (27.97)	18.6 (25.55)	624.3
27	11.64	5.85	0.00	0.00	23.07	18.79	18.5 (25.47)	18.5 (25.47)	622.1
30	11.63	5.83	0.00	-0.01	22.85	18.78	17.8 (24.95)	18.5 (25.47)	625.3
Mean	10.07	4.75	0.37	0.19	21.42	12.79	46.1 (42.57)	13.7 (21.31)	545.0
SEd	0.137	0.064	0.008	0.004	0.318	0.255	0.688	0.172	5.101
CD (P=0.05)	0.280	0.131	0.017	0.008	0.652	0.524	1.411	0.354	10.466

EC- Electrical conductivity; Figures in the parenthesis indicate arcsine values.

Table 3: Effect of days after anthesis (DAA) on seed physiological characteristics of yard long bean cv. Arka Mangala.

Days after anthesis (DAA)	Germination (%)	Speed of germination	Root length (cm)	Shoot length (cm)	Dry matter production (g/10 seedlings)	Vigour index I	Vigour index II
15	35 (36.27)	3.1	15.5	12.3	0.565	973	20
18	72 (58.05)	3.6	17.8	14.9	0.745	2354	54
21	80 (63.43)	5.2	21.6	18.7	0.853	3224	68
24	94 (75.82)	7.1	23.5	20.5	0.949	4136	89
27	94 (75.82)	7.1	23.4	20.5	0.940	4127	88
30	93 (74.66)	7.0	23.5	20.4	0.945	4083	88
Mean	78 (64.01)	5.5	20.9	17.9	0.833	3150	68
SEd	1.163	0.061	0.243	0.299	0.015	27.464	1.083
CD (P=0.05)	2.443	0.129	0.510	0.629	0.031	57.700	2.276

Figures in the parenthesis indicate arcsine values.

accordance with Adams and Rinne (1980). Table 2 shows that seed growth rate was rapid from fertilization to 9 DAA due to more water uptake, nutrients and also accumulation of photosynthates and the maximum growth rate of length (1.32 cm) and breadth (0.71 cm) was observed at 9 DAA , after that it reduced gradually and completely ceased.

Physiological and biochemical changes of seed during development and maturation

Germination capacity is the prime indicator of seed quality (Khan, 1977) and the final produce will become seed only after gaining capacity for regeneration. It was observed that developing seed started to germinate at 15 DAA to an extent of 35 per cent and the germination percent increased significantly with age of pods and reached maximum germination per cent (94%) at 24 DAA, but there was no significant different for seeds harvested at 24 DAA to 30 DAA (Table 3). Table 3 shows that the maximum germination per cent (94%) and speed of germination (7.1) of the seeds was coincided with maximum dry weight at 24 DAA and this was due to seed membrane organization as well increases in enzyme synthesis. Similar trend of germination was observed in mungbean by Anurag *et al.* (2009).

Woodstock and Combs (1964) coined root length and shoot length is a measure of seedling vigour because they reveal the performance of the seed under given environmental conditions. It was found that seedling length increased with advances in maturation and the maximum seedling length was attained at 24 DAA, which was coincided with maximum dry weight of seed and seed germination. Table 3 shows that dry matter production increased gradually from 15 DAA to 24 DAA and thereafter dry matter decreased slightly due to the development of inbuilt mechanism that involved in the disorganization of cell organelles after physiological maturity. Similar finding was also reported in peas by Mathews (1973). The computed vigour index of the present study was maximum at 24 DAA which was positively correlated with maximum dry weight of the seed. During seed maturation, protein content showed increasing trend and reached maximum (18.6%) at 24 DAA which was coincided with maximum dry weight of the seed (Table 2).

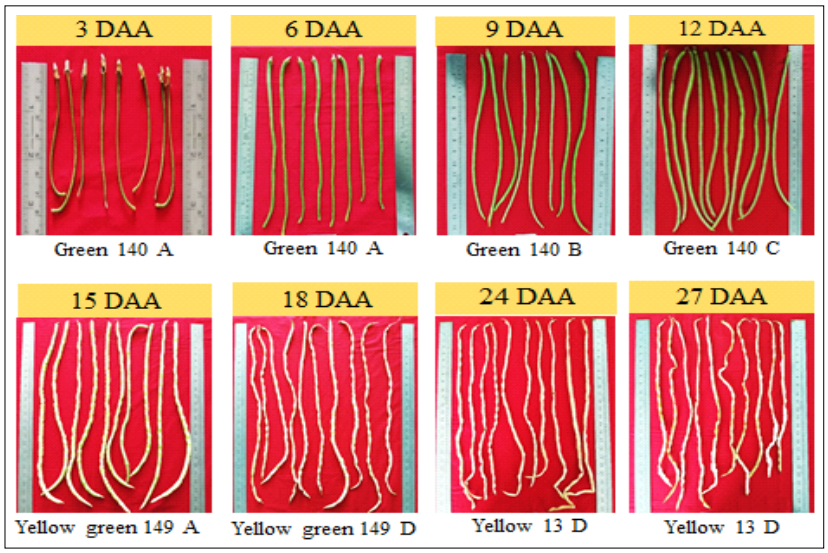

Plate 1: Pod development stages

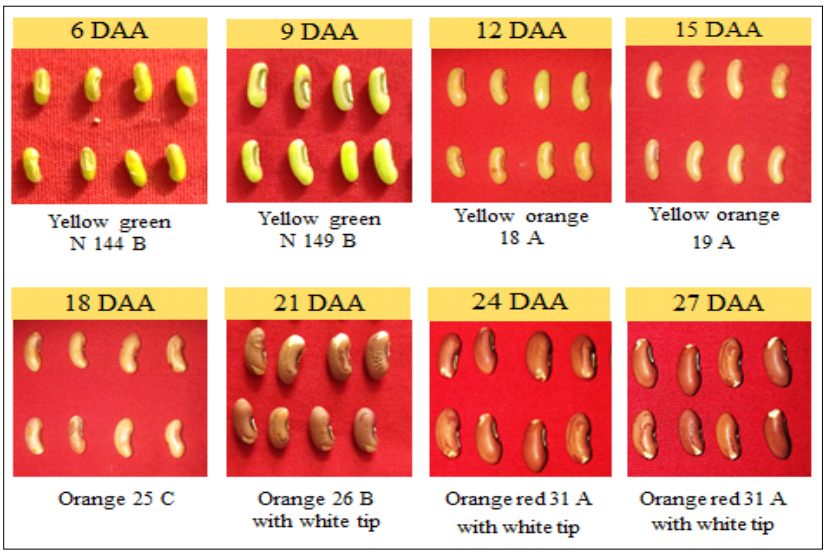

Plate 2: Seed development stages

The leachate conductivity of seeds increased with increase of maturity up to 18 DAA. After that decreasing trend was observed with increasing maturity and reached stability at 24 DAA. Table 2 indicates that the seeds attained its membrane stability at 24 DAA (624.3 μSm^{-1}) which was correlated with maximum germination capacity of seeds. Hosamani *et al.* (2012) in okra seeds also observed the increased trend of leachate conductivity because low quality seeds have poor membrane structure that allows the outward diffusion of ions during imbibition and it became stable at physiological maturity of seeds.

Pod and seed colour is the visual index of seed maturation. Carlson (1973) expressed that vascular system of the integumentary was destroyed as the seed mature, which coincided with the turning of seed colour. In the present study (Plate 1 and 2), it was observed that pod colour changes from green group (140 A) at 3 DAA to yellow group (13 D) at 24 DAA and the seed colour changes from green group (143 C) at 3 DAA to orange red group (31 A) with white tip at 24 DAA. The colour indicated was based on Royal Horticultural Society (RHS) colour chart. Yellow group (13D) of pods and orange red group (31A) with white tip of seeds at 24 DAA indicates the optimum time for harvesting seeds at physiological maturity with maximum seed quality.

CONCLUSION

Due to increasing human population, it is necessary to cultivate crop with high productivity along with nutritive protein to meet out the human demand. Hence, yard long bean satisfying these requirements was taken for the study to serve high quality seeds. Thus the present study on seed development and maturation revealed that seeds attained physiological maturation at 24 DAA with moisture content of 22% and the indices for maturity were maximization of

seed dry weight (18.80 g/100 seed), higher germination per cent (94%) and maximum seedling vigour. The visual indices for maturation was turning of pod to straw yellow colour and the seed to orange red group (31A) with white tip. Hence for high quality seeds, it is recommended that optimum date for harvesting pods was 24 DAA based on seed yield, germination capacity of seed and seedling viguor.

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