



Assessment of Physiological Maturity through Pod and Seed Developmental Pattern in Local Land Races of Green Gram (*Vigna radiata* L. Wilczek)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

For assessing physiological maturity through pod and seed developmental patterns in local landraces of green gram (*Vigna radiata* L. Wilczek), a sufficient number of flowers were tagged on the day of anthesis for all the genotypes. Ten fruits were harvested per replication for each genotype of five harvesting stages starting from 7 days after anthesis and at seven-day intervals. The harvested pods and seeds were utilized for the study. Enhancement in pod length, weight, no of seed/pod and 1000 seed weight was noted up to 28 DAA in most of the genotypes. Continuous increase in seed fresh weight was noted up to 28 DAA whereas dry weight continued to increase till 35 DAA in correspondence with loss in moisture. Maximum dry matter accumulation was between 14 DAA-21 DAA. Significant moisture loss was recorded for all genotypes from 21 DAA-35 DAA. The highest germination was achieved at 35 DAA for all genotypes except V1 and V4 for which the highest germination was recorded at 28 DAA. Enhancement in shoot length was up to 35 DAA for all genotypes whereas for root length it was till 28 DAA for most of the genotypes. Vigour Index 1 was noted to be constantly enhanced with progress in development up to 35 DAA for most of the genotypes whereas an increase in vigour index II was 28 DAA except V5. Germination and vigor index decreased after 28 DAA in 2 genotypes and 35 DAA for 3 genotypes indicating attainment of physiological maturity between 28-35 DAA.

Keywords: Green gram; seed development pattern; Days After Anthesis (DAA); vigor index; physiological maturity; genotypes.

1. INTRODUCTION

Greengram (*Vigna radiata* L. Wilczek) belongs to the family leguminosae and sub family papilionaceae, and has been grown as one of the principal crop for ages in India. Being the third largest pulse crop in India, it occupies 4.26 Mha in kharif and rabi with a production of 2.01 Mt at and yield level of 472 kg/ha during 2017-18. In Odisha, during the same year, it was grown in an area of 2.21 lakh hectares with an annual production of 0.80 lakh tonnes with a contribution of 5.42% in area and 4.22% in production (Ministry of Agriculture, GOI, 2018). Greengram is a short-day, self-pollinated, warm-season crop grown mainly in the semi-arid to sub humid tropics and tropics with 600 to 1000 mm of annual rainfall. It requires 22 to 35°C mean temperature during crop production and elevations not exceeding 1800 to 2000m above mean sea level (Srivastava et al., 2024). Green gram production is constrained by low soil moisture levels due to high temperatures caused by climate change, with average yields ranging from 300-700 kg/Ha despite the high potential of 1250 kg/Ha to 3000 kg/Ha obtained in trials. Low yields in green grams are caused by high temperatures, among other factors that result from increased solar radiation reaching the earth's surface (Mwangi et al., 2024; Patra et al., 2024). The difficulty of obtaining seeds of good physiological quality is among the limiting factors in the production of green gram. To express its full potential, the harvest must occur at the ideal

moment, with maximum dry matter accumulation, reaching high germination and vigor potential (Nogueira 2014). The seed development process is genetically controlled and involves an organized sequence of physical, biochemical, physiological and morphological changes, from fertilization until its independence from the plant, these changes also include a set of preparatory steps for the process of germination, which are characterized by the synthesis and accumulation of nutrient reserves (Filho et al., 2015). Seed vigour and viability reach a peak at physiological maturity (Ching et al., 1972; Andrews, 1966; Trammeli, 1983). In pulses, the development of seeds may start only after the fruit wall has grown to a large extent (e.g. Pea and Chickpea) or both seed and wall grow simultaneously (i.e. greengram and cowpea). A precise determination of the physiological maturity stage permits an accurate measure of the duration of the seed-filling period ensuring good seed quality. Information on the time required to attain Physiological Maturity, vigour and the time gap between Physiological Maturity and harvest maturity is useful to protect the seeds against deterioration as early or delayed harvesting of seed results in poor seed quality. However, currently, for Greengram, there is little information on the maturation and the ideal harvest period of the seeds, justifying the need to perform this evaluation. With this background, the present investigation was carried out to optimize harvesting time for five green gram cultivars at differing harvest dates.

2. MATERIALS AND METHODS

The experiment was performed at Experimental Farm, ICAR- CIWA, Bhubaneswar during *Rabi*, 2021 to study three locally collected genotypes and two advanced lines of green gram (Table-1) through morphological and growth parameters, to assess the physiological pattern of pod and seed development. The field experiment was laid out in randomized block design (RBD) with four replications in plots (size- 5 x 4 sq.m). Spacing between two consecutive plants was 20 cm and two rows was 30 cm. Recommended intercultural practices were followed. All five genotypes of green gram were critically observed from seedling emergence till they attained maturity. For studies on fruit and seed development, sufficient numbers of flowers were tagged on a particular blooming date of all the genotypes to avoid hazards/variation due to environmental influence. Developing fruits were harvested on each of five different stages starting from 7 days after anthesis (DAA) and at 7 days intervals thereafter, viz., Stage-I: 7 DAA, Stage-II: 14 DAA, Stage-III: 21 DAA, Stage – IV: 28 DAA and Stage – V: 35 DAA. At each stage, 10 fruits from each of four replications for all the genotypes were sampled. The seeds were separated carefully from the fruit wall. Observations on various fruit and seed characters were recorded on randomly harvested ten (10) fruits at each stage for each genotype per replication so that symmetry in the methodology to the maximum extent is achieved. The fruit and seed characters observed were pod length (mm), pod weight (mg), number of seeds per pod, thousand (1000) seed weight (gm), seed fresh and dry Weight (mg), seed moisture content (%), seed germination (%), seedling root-shoot length (cm) and seedling dry weight (mg), seed vigour index (SVI), total protein content of seed. The germination test was carried out using germination papers by the between-paper (BP) method (ISTA, 1995) for all the stages of five genotypes. The seeds collected for five (5) successive stages were considered for germination test. The final count was taken on 8th day. Percentage protein was determined with the help of Nitrogen determination by Kjeldhal method.

3. RESULTS AND DISCUSSION

ANOVA (Analysis of Variance) revealed significant differences among the genotypes, stages of development and their interaction for most of the characters studied. The farmer's

variety (GV-3) exceeded other genotypes in all characteristics except seed vigour index-II where the maximum value was observed by genotype-3 (OBGG-102).

3.1 Growth Parameters

Genotypes exhibited more than 100% increase in weight in the earlier days i.e. during the second week. The active growth rate was recorded from 7 DAA to 21 DAA. After 21 DAA, gain in pod weight started being negative up to 35 DAA for all genotypes. A number of seeds per pod were recorded in 4 developmental stages from 14 DAA to 35 DAA as at 7DAA, the seed growth was not sufficient enough for evaluation. The highest individual increase was recorded in V5 (106.06%) from 14 to 21 DAA (Fig. 3). Average 1000 Seed Weight was the maximum for genotype 5 (36.78g) followed by genotypes 2 and 1. Genotype V4 exhibited the lowest mean 1000 Seed Weight and also across all stages. 1000 Seed Weight was maximum at 21 DAA for all genotypes except in V5 which attained maximum weight at 28 days (Fig. 4). This varying trend indicates that 1000 seed weight is primarily controlled by the individual genetic background of the genotypes. Enhancement in pod length, weight, no. of seeds/pod and 1000 seed weight was noted up to 28 DAA in most of the genotypes. Enhancement was maximum from 7 DAA to 21 DAA. Pod characters along with seed size and weight show a direct relationship with physiological quality in advanced stages. Bold seeds were better nourished during their development, had well-formed embryos and a greater amount of reserves, with greater potential for germination and more vigorous plants when compared to smaller seeds (Carvalho and Nakagawa 2012). Observation showed that as the harvesting was delayed, more seeds were filled and consequently, average seed weight increased. All these findings are in agreement with the findings of Nimje and Gandhi ,1994; Siddique and Wright, 2003; Anurag et al.,2009).

3.2 Physiological Parameters

For understanding the stage of physiological maturity Dry matter accumulation, moisture content and vigour index were of prime importance. An increase in seed fresh weight was noticed with the advancement of seed development stages. The average weight of fresh seed was recorded to be maximum in stage III i.e., 21 DAA and decreased during subsequent

stages. The increase in fresh weight of seeds with the advancement of seed development stages was documented by Varshney et al. (2001) in Indian mustard, and Mahesha et al. (2001) in sunflower, Moonmoon and Ahmad (2020) in Country beans.

Table 1. Details of the genotypes

Sl.no	Type of Genotype	Name of genotype	Source (Place of collection)
V1	Farmer's variety	Nayagarh Local Green	Hariharpur, Nayagarh, Odisha
V2	Advance line	OBGG-101	OUAT, Bhubaneswar
V3	Advance line	OBGG-102	OUAT, Bhubaneswar
V4	Farmer's variety	Nayagarh Local Black	Godipally, Nayagarh, Odisha
V5	Farmer's variety	GV-3 (P-3)	OUAT, Bhubaneswar

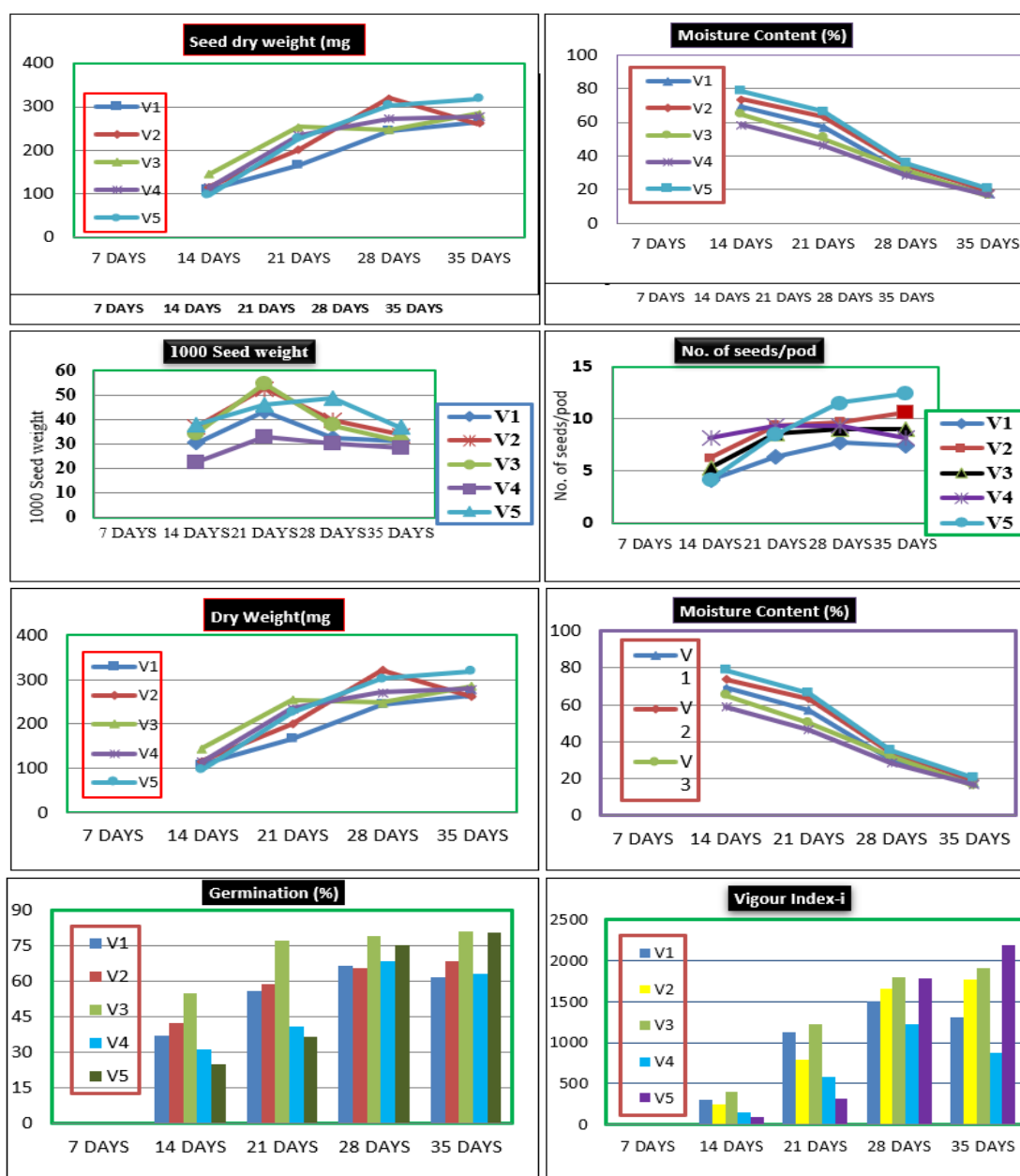


Fig. 1-8. Changes in seed developmental parameters at different maturity stages from 7 to 35 DAA

Table2. Pod and seed development parameters at different maturity stages from 7 to 35 Days After Anthesis (DAA) for 5 different genotypes of Greengram

Genotypes	DAA	Pod length (mm)	Pod weight (mg)	No of seed/pod	1000 seed weight (g)	Seed fresh weight(mg)	Seed dry weight (mg)	Seed moisture content (%)
V1	7	30.45	145.25	-	-	-	-	-
	14	46.03	508.00	4.11	30.27	370.15	108.45	69.13
	21	49.68	723.50	6.35	43.18	414.20	165.80	57.15
	28	59.63	455.00	7.71	32.65	360.38	243.93	30.10
	35	61.27	382.88	7.45	31.23	322.03	265.20	17.38
	Mean	49.41	442.93	6.41	34.33	366.69	195.84	43.44
V2	7	33.95	144.25	-	-	-	-	-
	14	49.28	572.00	6.23	37.06	433.65	116.15	73.80
	21	58.95	792.25	9.33	52.88	565.65	200.68	63.15
	28	62.03	453.25	9.64	39.93	482.50	320.78	34.08
	35	65.88	357.01	10.60	33.68	327.35	260.45	19.26
	Mean	54.02	463.75	8.95	40.89	452.29	224.51	47.57
V3	7	30.30	140.00	-	-	-	-	-
	14	35.98	367.25	5.33	34.29	423.65	145.58	64.83
	21	49.90	601.75	8.63	54.68	554.70	253.93	50.40
	28	56.75	436.00	9.04	37.69	382.90	248.13	31.93
	35	55.92	417.77	9.00	31.13	342.25	284.58	16.17
	Mean	45.77	392.55	8.00	39.45	425.88	233.05	40.83
V4	7	38.43	167.25	-	-	-	-	-
	14	45.28	362.00	8.23	22.35	304.15	115.63	58.80
	21	59.48	574.75	9.38	32.93	442.98	234.90	46.53
	28	67.53	508.00	9.36	30.08	381.78	271.55	28.43
	35	66.77	377.59	8.18	28.33	336.33	278.45	16.82
	Mean	55.49	397.92	8.78	28.42	366.31	225.13	37.64
V5	7	32.55	116.75	-	-	-	-	-
	14	49.28	515.00	4.13	37.92	443.05	95.55	78.45
	21	60.80	836.50	8.50	46.21	646.88	226.43	66.63
	28	68.98	524.50	11.58	48.73	443.45	301.78	35.40
	35	62.05	443.67	12.50	36.78	395.33	318.78	20.36
	Mean	54.73	487.28	9.18	42.41	482.18	235.63	50.21

Table 3. ANOVA data of physiological characters of 5 genotypes of Greengram

S.N.	Parameters	Anova value at different days after anthesis				
		F table value at 5% (3.260) and 1% (5.410) level of significance				
Mean sum of square		07 DAA	14 DAA	21 DAA	28 DAA	35 DAA
1	Pod length	3.993*	4.284*	11.458**	2.957	7.285**
2	No of seed/pod	2.227	4.232*	7.869**	1.070	10.712**
3	1000 seed weight		11.063**	17.724**	12.568**	14.210**
4	Seed fresh weight		33.947**	36.584**	23.349**	14.935**
5	Seed dry weight		14.365**	52.024**	10.039**	4.411*
6	Seed moisture content		9.662**	14.813**	6.605**	4.724*
7	Pod weight		861.512**	964.035**	263.512**	1569.413**
8	Seed Germination		23.805**	69.153**	11.142**	72.441**
9	Seedling shoot length		12.054**	88.707**	3.444*	37.797**
10	Seedling root length		2.176	7.795**	10.336**	30.186**
11	Seedling length	36.621**	14.161**	132.953**	13.122**	36.621**
12	Vigour Index I	15.710**	16.167**	42.431**	15.676**	15.710**
13	Vigour index II	44.207**	10.940**	25.928**	56.405**	44.207**

** significant at both 1% and 5% level, * significant at only 5% level, No star means non significant

The attainment of maximum fresh weight (Fig. 4) of seed indicated the cessation of cell division during seed maturity (Noggle and Fritz, 1991). Maximum Seed dry weight (Fig. 5) in different developmental stages was varied in different genotypes (V5 highest at 35 DAA; V2 at 28 DAA; V3 at 14,21 DAA). The average weight of dry seed was recorded to be maximum in stage V i.e., 35 DAA. The active dry matter accumulation period was from 14 to 21 DAA. Results (Fig. 6) revealed that the differences in moisture content become less in advanced growing stages and genotypes tend to attain similar moisture levels towards physiological maturity. The average moisture content of the seed was recorded to be maximum in stage II i.e., 14DAA (69.00%). Similar behavior was described by Eskandari (2012) in seeds of *Vigna sinensis* (2012) in common bean seeds Nogueira et al. (2014) and Cruz et al., 2019 in cowpea seeds. Seed moisture content was (Fig. 6) minimum at 35 DAA for all genotypes. Maximum dry matter was accumulated and maximum loss of seed moisture was recorded between 28 to 35 DAA. The increase in dry weight of the seeds towards maturity reflects the accumulation of non-structural carbohydrates, reducing sugars and fibre, relative to seed moisture content which showed a sharp fall (Ketsa and Poopattarangk, 1991). At 28 DAA the genotypes reached nearly 30% moisture with 43% reduction over 21 DAA. Moisture was further reduced by 44% DAA to 35 DAA keeping it to 18%. The decrease in moisture content with the advancement of the maturity stage might be due to desiccation and dehydration. Loss of seed moisture during

ripening and maturation of a seed is a common phenomenon and has been observed in many crops (Karivaratharaju, 1974). Similar reduction in moisture content during maturation was reported by Mahesha et al., 2001 in sunflower; Varshney et al., 2001 in Indian mustard; Samnotra et al., 2002 in okra; Khatun et al., 2009 in Lentil Geetha et al., 2013 in mustard; Nichal et al, 2018 in soybean; Cruz et al., 2019 in cowpea and Moonmoon and Ahmad (2020) in country bean.

3.3 Seed Germination

Initiation of germination was recorded at 14 DAA which increased to 54% at 21 DAA. When consideration was made on an individual genotype basis, germination with the highest magnitude (81%, 80.75% & 68.75%) was recorded for the genotypes 3,5 and 2 at 35 DAA (Fig. 7). average germination was recorded to be maximum in stage IV i.e., 28DAA (71.30%). Maximum rate of increase was exhibited by all genotypes from 14 DAA to 21 DAA with a range of 143.64% to 217.86%. The lowest germination percentage was observed in II stage. It indicated that germination was markedly less in early-harvested seeds compared to seeds harvested at later stages. Lesser germination (%) in early harvested seeds may be due to the presence of more immature and unfilled seeds. (Khatun et al., 2009). Similar results have been reported (Oliveira et al., 1999; Demir and Ellis, 1992; Siddique and Wright, 2003) that physiological maturity does not coincide with maximum germination which may be a result of dormancy factor associated with fruit sap or any relation

between activation of germination inducing enzymes with decreasing seed moisture content, Shoot length of all genotypes reached maximum length at 35 DAA except V1 where maximum shoot length reached 21 DAA after which subsequent reduction in length was observed in further stages. The root length of genotypes 1, 2 and 4 reached maximum at 28 DAA whereas, for genotypes 3 and 5, the longest root was recorded at 35DAA. Maximum elongation in both root and shoot length was recorded from 14 DAA to 21 DAA with a maximum rate of shoot elongation in V4 (215.91%) and root elongation in V5 (215.52%). The total seedling length was increased for all genotypes with a decreased rate of elongation up to 35 DAA except genotype 1 where it became negative after 28 DAA indicating physiological maturity. Cruz et al., 2019 observed maximum root and shoot length value in cowpeas at the last harvest, at 21 days after anthesis, which is due to the metabolic and catabolic events of accumulation in the reserves tissue throughout the development of the seed.

When consideration was made on an individual genotype basis, vigour index-I with highest magnitude (2184.98, 1911.43, 1770.45) was recorded for genotypes 5, 3 and 2 respectively at 35DAA and for genotype 1 (1507.95) and 4 (1221.20) at 28DAA maturity (Fig. 8). Average vigour index-I was recorded to be maximum in stage V i.e., 35DAA (1610.97). Maximum rate of increase was exhibited by all genotypes from 14 DAA to 21 DAA with a range of 489.13% to 622%. The rate continued to increase till 35 DAA for genotypes 2, 3 and 5 whereas it became negative for genotypes 1 and 4 after 28 DAA. When individual genotypes were considered, maximum seed Vigour index –II was recorded for all the genotypes at 28 DAA except V5 where maximum vigour index was recorded at 35DAA. The maximum rate of increase was exhibited by all genotypes from 14 DAA to 21 DAA with maximum in genotype 1 (220.22%) and minimum in genotype 3 (107.40%). After 28 DAA, the rate of increase stopped and became negative afterwards except V5 where a further increase in vigour was recorded. Maximum decrease in vigour index after 28 DAA was in genotype 4 (-11.21%). Vigour Index I, 98% increase from 21 DAA-28 DAA, which was similar at 35 DAA. However, Vigour Index II showed an increase till 35 DAA which was 35% over 28 DAA. higher seed vigour index-II probably due to the associative effect of germination percentage and seedling length (Khatun et al., 2009; Eskandari., 2012). Increased seed vigour index-II might be

due to the maturation of seeds in later stages resulting in an improvement of germination percentage and seedling length. Khare and Satpute (1999) reported similar results. Germination and Vigour Index decreased after 28 DAA for Nayagarh Local Green and Nayagrh Local Black indicating attainment of Physiological Maturity at 28 DAA. For OBGG-101, OBGG-102 and GV-3 Physiological Maturity was inferred to be attained at 35 DAA. Seeds developed in different species generally attain maximum germination and vigour at the end of the seed-filling period, when physiological maturity is reached (Welbaum, 1999). However, in some those species, maximum seed dry matter accumulation and seed quality do not coincide, such as pepper (*Capsicum annum* L.) (Demir and Ellis, 1992b; Oliveira et al., 1999), melon (*Cucumis melo* L.)(Welbaum and Bradford, 1988) and tomato (*Lycopersicon esculentum* Mill.)(Kwon and Bradford, 1987; Berry and Bewley, 1991; Demir and Ellis, 1992a; Demir and Samit, 2001; Dias et al., 2006 a). In tomato seeds, Kwon and Bradford (1987) found that maximum germination and vigour occurred 15 days after the attainment of maximum dry mass. It was also verified by Demir and Ellis, 1992a, to whom the highest germination percentage was obtained at 70 DAA, while the maximum dry matter content occurred at 50 DAA. Then, the occurrence of maximum seed quality during development and its association with seed and fruit features are important factors in defining the ideal harvest time (Botelho et al. 2016).

4. CONCLUSION

For understanding the stage of physiological maturity Dry matter accumulation, moisture content and vigour index were of prime importance. Maximum dry matter was accumulated and maximum loss of seed moisture was recorded between 28 to 35 DAA. Germination reached maximum at 28 DAA which was at par with 35 DAA for all genotypes. In Vigour Index 1, 98% increase from 21 DAA-28 DAA, which was similar at 35 DAA. However, Vigour Index 2 showed an increase to 35 DAA which was 35% over 28 DAA. Germination and Vigour Index decreased after 28 DAA for V1 and V4 indicating attainment of Physiological Maturity at 28 DAA. For V2, V3 and V5 Physiological Maturity was inferred to be attained at 35 DAA.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models

(ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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