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Identification of viable immature seed stage for accelerating breeding cycle in red gram (*Cajanus cajan*)

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Abstract

Red gram (*Cajanus cajan*) is an important pulse crop, but its improvement through conventional breeding is slow due to the long generation time. Early seed harvest before physiological maturity can accelerate breeding cycles in speed breeding programs. The present study aimed to identify the stage at which red gram seeds attain viability before physiological maturity. Immature pods of cv. CO (Rg) 7 were harvested at 27-40 days after anthesis (DAA) and evaluated for seed quality parameters. Seed viability reached 100% from 29 DAA onwards, while germination and seedling vigour improved with maturity. Although maximum vigour occurred at 36 DAA, seeds harvested at 31-33 DAA were suitable for early generation advancement.

Keywords: Speed breeding, red gram, early harvest

Introduction

Red gram (*Cajanus cajan*) is an important legume crop widely cultivated in tropical and subtropical regions, particularly in India, where it plays a crucial role in nutritional security and soil fertility through biological nitrogen fixation. India is the largest producer and consumer of red gram globally, contributing approximately 25-28% of the total pulse production worldwide. The crop is predominantly cultivated in states such as Karnataka, Maharashtra, and Telangana [1, 2, 3, 4]. In India, red gram is grown over an area of about 3.89 million hectares with a production exceeding 3.3 million tonnes and an average productivity of approximately 800-850 kg ha⁻¹, although productivity varies across regions. Among the major producing states, Karnataka accounts for nearly 0.83 million hectares with an average productivity of around 734 kg ha⁻¹ [1, 4].

Development of improved cultivars through conventional breeding approaches is a time-consuming process, often requiring more than a decade to release a new variety. This long breeding cycle limits the ability to rapidly respond to increasing food demand and challenges posed by climate change [5, 6]. Therefore, accelerating breeding cycles has become essential for the rapid development of high-yielding, disease-resistant, and climate-resilient cultivars. Speed breeding has emerged as an effective approach to shorten generation time by enabling multiple crop cycles within a year. This technique utilizes controlled environmental conditions such as extended photoperiods, optimized light intensity and quality, temperature regulation, and early seed harvest to accelerate plant growth and reproduction [7, 8, 9, 10]. Among these approaches, early harvesting of seeds before full physiological maturity is a promising strategy for reducing the crop cycle duration while maintaining seed viability and vigour.

Seed viability is often attained prior to physiological maturity in several crops. For instance, [11] reported that in *Physalis angulata*, seeds became viable at 21 days after anthesis (DAA), whereas physiological maturity occurred at 35 DAA, indicating that seed viability can precede full seed maturation. Identifying the stage at which seeds become viable is therefore critical for implementing early seed harvest strategies in speed breeding programs.

However, limited information is available regarding the stage at which red gram seeds attain viability prior to physiological maturity. Therefore, the present study was undertaken to determine the stage at which red gram seeds become viable before physiological maturity.

Materials and Methods

Experimental location and plant material

The experiment was conducted at the Department of Seed Science and Technology, Tamil Nadu Agricultural University (TNAU), Coimbatore, India. The red gram (*Cajanus cajan*) photo-insensitive variety CO (Rg) 7 was raised in Field No. 4A of the New Area, TNAU farm. Standard agronomic practices recommended for red gram cultivation were followed during the crop growth period.

Flower tagging and collection of immature seeds

Freshly opened flowers were tagged on the day of anthesis to monitor seed development stages. Immature pods were harvested at different stages ranging from 27 to 40 days after anthesis (DAA). The collected pods were used to record physical and physiological seed quality parameters such as fresh weight of immature pods, fresh weight of immature seeds, speed of germination, germination percentage, seed viability percentage, root length, shoot length, and vigour index.

Evaluation of Seed Quality Parameters

Speed of germination

The speed of germination was calculated using the standard procedure based on daily germination counts and expressed as an index reflecting the rate of germination.

Germination percentage

The germination test was conducted following the procedure outlined by ISTA (1999) using the paper method. Four replications of 100 seeds each were used for the test. Seeds were placed in a germination room maintained at 25 ± 2 °C temperature and $90 \pm 3\%$ relative humidity. At the end of the 10th day, the number of normal seedlings in each replication was counted and the germination percentage was calculated based on the average number of normal seedlings [12].

Root length (cm)

Ten normal seedlings were selected randomly from each replication and the root length was measured from the collar region to the tip of the primary root using a scale and expressed in centimetres.

Shoot length (cm)

Ten normal seedlings were randomly selected from each replication and the shoot length was measured from the collar region to the tip of the primary leaf and expressed in centimetres.

Dry matter production

The ten seedlings used for measuring seedling length were shade-dried initially and then oven-dried at 85 ± 2 °C for 24 hours in a hot air oven. The dried seedlings were cooled in a desiccator for 30 minutes and weighed. The dry matter production was expressed in grams per ten seedlings.

Vigour index

The vigour index was calculated using the formula proposed by Abdul-Baki and Anderson [13]

Vigour Index = Germination (%) $\times$ Mean seedling length (cm)

Results

Immature pod weight, immature seed weight and seed viability varied significantly among the harvest stages (27-40 DAA). The immature pod weight was highest at 27 DAA (13.28 g) and progressively decreased with advancement in days after anthesis, reaching the lowest value at 40 DAA (3.98 g). The mean immature pod weight recorded was 6.141 g with SED 0.117 and CD ($P = 0.05$) 0.24, indicating significant differences among the stages.

Immature seed weight increased initially from 1.82 g at 27 DAA to a maximum of 2.01 g at 29 DAA, followed by a decline and slight stabilization in the later stages. The seed weight ranged between 1.00 g and 1.20 g from 30 to 37 DAA, and slightly decreased thereafter to 1.09 g at 40 DAA. The mean immature seed weight was 1.295 g with SED 0.021 and CD ($P = 0.05$) 0.043, showing significant variation among harvest stages.

Seed viability increased with the advancement of maturity. Seeds harvested at 27 and 28 DAA recorded 90 per cent viability, whereas 100 per cent viability was observed from 29 DAA onwards. The mean seed viability was 98.571 per cent, with SED 0.617 and CD ($P = 0.05$) 1.264.

The attainment of 100 per cent seed viability from 29 DAA onwards indicates that seeds harvested at this stage are physiologically capable of germination. Hence, 29 days after anthesis can be considered as the earliest suitable harvest stage for seed extraction in speed breeding of red gram, enabling reduction in generation time while maintaining seed viability (Table 1.).

Germination percentage increased progressively with advancing maturity. The lowest germination was observed at 27 DAA (~40%), which increased to about 56% at 29 DAA and further improved steadily, reaching around 60-66% between 31 and 40 DAA, with the highest germination recorded at 38-40 DAA.

Root length also showed variation among harvest stages. The maximum root length was recorded around 36 DAA (~19 cm), while relatively lower root lengths were observed at 29-31 DAA (~16 cm). In the later stages (38-40 DAA), root length remained moderately high (~17-18 cm).

Shoot length was highest at 27 and 36 DAA (~17 cm), whereas lower shoot length was observed at 29 DAA (~11 cm). In the subsequent stages, shoot length ranged between 13-16 cm, indicating stabilization of seedling growth with advancing maturity.

The speed of germination showed moderate variation across harvest stages. Higher values were observed at 27 DAA (~2.3) and 31-37 DAA (~1.6-1.7), whereas slightly lower values were recorded at 33 and 40 DAA.

The vigour index increased markedly with advancement in maturity. The lowest vigour index was observed at 27 DAA (~1500) and 29 DAA (~1520), after which it increased substantially, reaching a maximum at 36 DAA (~2200). In the later stages (38-40 DAA), the vigour index remained relatively high (~1950-2050).

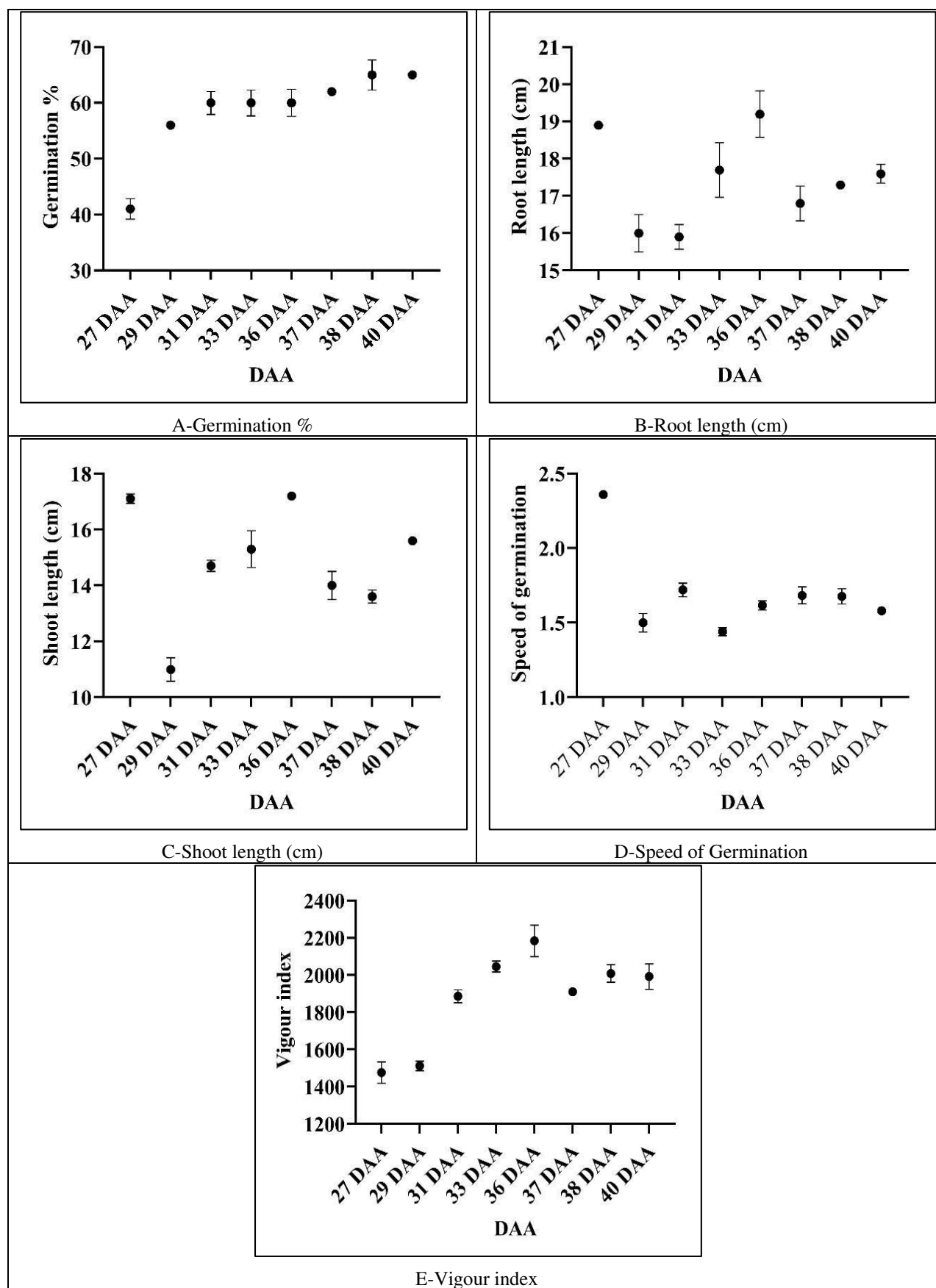


Fig 1: Seed quality parameters of red gram cv. CO (Rg) 7 seeds at different days after anthesis

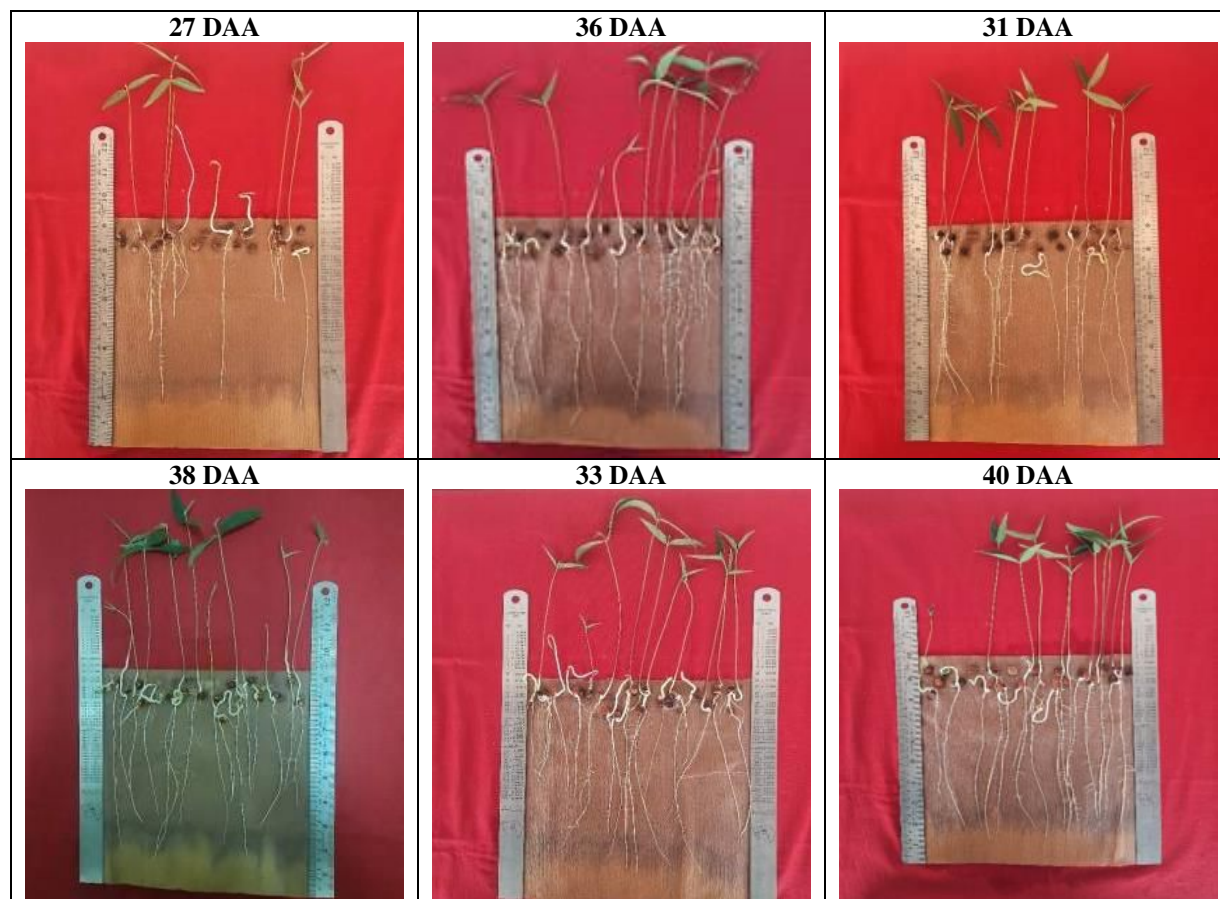


Fig 2: Germination (%) of red gram cv. CO (Rg) 7 at different days after anthesis

Table 1: Identification of the stage of acquisition of embryo viability during seed development and maturation

DAA	Immature pod weight	Immature seed weight	Seed viability %
27	13.28	1.82	90
28	12.15	1.91	90
29	8.11	2.01	100
30	7.40	1.00	100
31	4.95	1.03	100
32	4.77	1.17	100
33	6.10	1.14	100
34	4.11	1.10	100
35	4.53	1.13	100
36	4.25	1.20	100
37	4.20	1.20	100
38	4.09	1.18	100
39	4.05	1.15	100
40	3.98	1.09	100
Mean	6.141	1.295	98.571
SEd	0.117	0.021	0.617
CD	0.24	0.043	1.264

Discussion

In legumes like green gram and horse gram, physiological maturity typically occurs between 28 to 49 days after anthesis (DAA), when seed dry weight peaks and vigor indices are highest [14, 15]. Germination percentage of seeds harvested at different days after anthesis (DAA) generally increases as seeds mature, reaching a peak near physiological maturity. For example, in green gram, seeds harvested before 30 DAA did not germinate, while those harvested at 40 DAA showed the highest germination potential around 81% [16]. Similarly, in African eggplant,

seeds from fruits harvested at 30 DAA had very low germination, but germination improved significantly with after-ripening and reached maximum levels (78-96%) when harvested at 60-70 DAA [17]. For cowpea, seeds attain physiological maturity and maximum viability at about 35 DAA, with high vigor observed at 45 DAA; seeds harvested earlier may germinate but often fail to develop into vigorous seedlings [18]. In *Campomanesia adamantium*, seed viability and vigor peak between 52 and 70 DAA, with a marked decline after 70-75 DAA [19]. Speed breeding protocols use controlled environments with extended photoperiods and temperature regulation to accelerate flowering and seed development, allowing early seed harvest without compromising seed viability [20, 8]. For example, in pea (*Pisum sativum*), early grain harvest combined with a hydroponic system and controlled light conditions enabled up to five generations per year, significantly reducing cycle time and costs [21]. In chickpea, immature seeds harvested as early as 20-23 days after anthesis were successfully germinated, allowing up to seven generations annually under extended photoperiods [22].

Conclusion

Seeds harvested at 36 DAA exhibited superior seedling vigour, higher root length and maximum vigour index, indicating better physiological maturity. However, considering early generation advancement in speed breeding, seeds harvested around 31-33 DAA may be considered suitable, as they recorded acceptable germination and seedling vigour while reducing the generation time compared to later harvest stages.

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