



# GA<sub>3</sub> acts as a germination promoter in wild castor bean (*Ricinus communis* L.)

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## Abstract

In this study, we focused on overcoming germination inability in wild castor bean by treatment with different concentrations of gibberellic acid (GA<sub>3</sub>) and potassium nitrate (KNO<sub>3</sub>). The caruncle of mature seeds of wild castor bean, collected from natural habitats in Türkiye, were removed at the seed tip. Seeds with or without caruncle were immersed in GA<sub>3</sub> and KNO<sub>3</sub> at concentrations of 0, 250, 500 and 1000 ppm. Germination and seedling growth parameters were evaluated. Removing the caruncle from the seeds significantly improved germination (from 14.7 to 73.5%) and seedling growth characteristics. Application of KNO<sub>3</sub> to the seeds without caruncle shortened mean germination time. GA<sub>3</sub> promoted root elongation, but shoot growth was induced by KNO<sub>3</sub>. Hydration had a stimulative effect on seedling fresh weight and dry matter, while the highest vigour index was identified in seeds primed with GA<sub>3</sub>. The beneficial effects of KNO<sub>3</sub> and GA<sub>3</sub> were maximal when treated with 500 ppm and 1000 ppm, respectively, while higher germination and better seedling growth were obtained from hydration compared to KNO<sub>3</sub>. It is suggested that the presence of the caruncle restricts germination and 1000 ppm GA<sub>3</sub> acts as a germination promoter in wild castor bean seeds.

**Keywords:** caruncle, dormancy, germination, gibberellic acid, potassium nitrate

## Introduction

Castor bean (*Ricinus communis* L.), an important industrial and medicinal plant, is a member of the *Euphorbiaceae* family. Although it has over 700 uses, from medicine and cosmetics to biodiesel, plastics and lubricants (Lopes *et al.*, 2018; Thiruppathi *et al.*, 2018), its main use is for oil with the seeds having 35 to 55% oil, rich in ricinoleic, linoleic, oleic and stearic acids (Chen *et al.*, 2004; Shafiee *et al.*, 2009). The seeds, leaves

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and stems of the castor plant contain alkaloids like ricin and ricinine which are poisonous to humans and animals, but is utilised for medicinal purposes (Pashazadeh and Başalma, 2012; Sousa *et al.*, 2022).

The castor plant grows in the wild in large quantities in most tropical and subtropical countries, and is cultivated in many countries throughout the world (Ogunniyi, 2006). It is native to the Ethiopian region of tropical East Africa and is globally grown on 1.2 million hectares of land, with a production of 2 million tons (Auld *et al.*, 2009; FAO, 2022). In Türkiye, the castor bean is found in natural vegetation throughout the Mediterranean region (TUBIVES, 2022), although it is not yet in cultivation. In 2021, Türkiye imported 10.2 thousand tons of castor oil, at a cost of 18.5 million dollars (TUIK, 2022). To meet castor oil demand, either a breeding programme for cultivation must be undertaken or the oil production must be met from the seeds collected from naturally growing plants.

A prerequisite for the cultivation of castor bean is rapid and synchronised germination, emergence and seedling establishment. However, the mature seeds of castor exhibit some degree of dormancy, manifested in slow, erratic and low germination, probably caused by hard seed coats, limiting water permeability (Msaakpa *et al.*, 2013). Several seed treatments have been useful for overcoming germination inabilities. Caruncle removal, for example (Martins *et al.*, 2006; Sousa *et al.*, 2009; Severino *et al.*, 2012), as well as soaking seeds in water for 24 hours or removing the caruncle and piercing the testa (Costa Nobre *et al.*, 2013; Salihu *et al.*, 2014), improved germination percentage. Improved germination following sulphuric acid scarification has also been reported (Sousa *et al.*, 2009). Furthermore, one of the most important applications for breaking dormancy and promoting germination is seed priming. It is a technique to control the water uptake process followed by re-drying, which allows the seeds to absorb water and induce enzymatic activities responsible for germination, but does not allow the seeds to actually germinate (Kaya, 2008; Ergin *et al.*, 2021; Awasthi *et al.*, 2022). Hydration and various substances such as gibberellic acid, kinetin, potassium nitrate and polyethylene glycol have been used to promote germination and seedling growth of seeds of several crop plants (Kaya *et al.*, 2006; Draganić and Lekić, 2012; Kaya and Kulan, 2020; Shokouhian and Omid, 2021). Our study concentrated on overcoming the germination inability of wild-sourced castor bean seeds by removing the caruncle and priming with different concentrations of gibberellic acid ( $GA_3$ ) and potassium nitrate ( $KNO_3$ ).

## Materials and methods

This study was conducted at the Seed Sciences Laboratory in the Department of Field Crops, Eskişehir Osmangazi University, Türkiye. The mature castor bean seeds were collected in late August 2021 from secondary racemes on a shrub-like perennial plant in Tömük, Erdemli/Mersin in the Mediterranean region of Türkiye. The seeds were manually threshed and stored at 4°C until they were used.

The seeds were separated into two groups. In the first group, referred to as “without caruncle”, the seed tip was removed by nail clippers; the seed tip was left in the other group, referred to as “with caruncle”. For priming, the seeds with or without caruncle

were soaked in different concentrations of GA<sub>3</sub> or KNO<sub>3</sub> (0, 250, 500 and 1000 ppm) at 20°C for 16 hours in the dark. Distilled water (0 ppm) was referred to as hydration in the study. Non-primed seeds served as a control. After the treatments, the seeds were surface-dried with paper towels and dried directly back to their initial seed weight at room temperature (22-24°C) for 2-4 days. To equilibrate seed moisture content of non-primed and primed seeds, they were left at room conditions for three days. The moisture contents of primed and non-primed seeds were between 7.2 and 7.6%.

The standard germination test was conducted using four replicates of 50 seeds. The 50 seeds were inserted in three-layer filter papers moistened with 7 mL of distilled water for each paper. After the papers were rolled, they were placed into sealed plastic bags to avoid moisture loss. The seeds were germinated in an incubator at 25°C in the dark. The seeds with radicle 2 mm were considered to be germinated, and they were counted every 24 hours for 14 days to calculate the mean germination time for evaluating germination speed, as described by ISTA (2003). The germination index (GI) was also computed according to the following formula.  $GI = \text{Number of germinated seeds/days of first count} + \dots + \text{Number of germinated seeds/days of final count}$  (Salehzade *et al.*, 2009).

Root length, shoot length and seedling fresh weight were measured using a ruler for five seedlings chosen randomly from each treatment on the 14<sup>th</sup> day. The vigour index was calculated by multiplying germination percentage (%) and seedling length (cm).

The data were analysed using the MSTAT-C computer program (Michigan State University v.2.10) in a completely randomised design with four replicates using a two-factor factorial. Data given in percentages were subjected to arcsine transformation before statistical analysis. The means were compared using the Least Significant Differences (LSD) value at  $P < 0.05$  level.

## Results

A significant difference between priming agents for the breaking of seed dormancy in castor was calculated for germination percentage, mean germination time and germination index ( $P < 0.05$ ). No germination and seedling growth were observed in control seeds (figure 1). Germination was positively influenced by the absence of the seed caruncle, priming agents and their doses. The mean germination percentage increased from 14 to 74% with the removal of the caruncle. Among the priming agents, GA<sub>3</sub> was superior to KNO<sub>3</sub>, and in both priming agents, the most effective dose for increasing germination was 1000 ppm (table 1). Application of 1000 ppm GA<sub>3</sub> to the without caruncle seeds improved germination percentage and index and vigour index (figure 1). Application of 250 or 500 ppm GA<sub>3</sub> to the seeds without caruncle reduced the mean germination time, and the slowest germination was achieved in the hydrated seeds without caruncle (figure 1). Mean germination time was not calculated in the seeds with or without caruncle applied by KNO<sub>3</sub> levels and control because no seeds germinated.

As with the germination percentage, the superiority of GA<sub>3</sub> was also revealed in the germination index (table 1). The seeds without caruncle treated with 500 ppm GA<sub>3</sub> had the highest germination index. It was promoted by each increase in KNO<sub>3</sub> levels (figure 1).

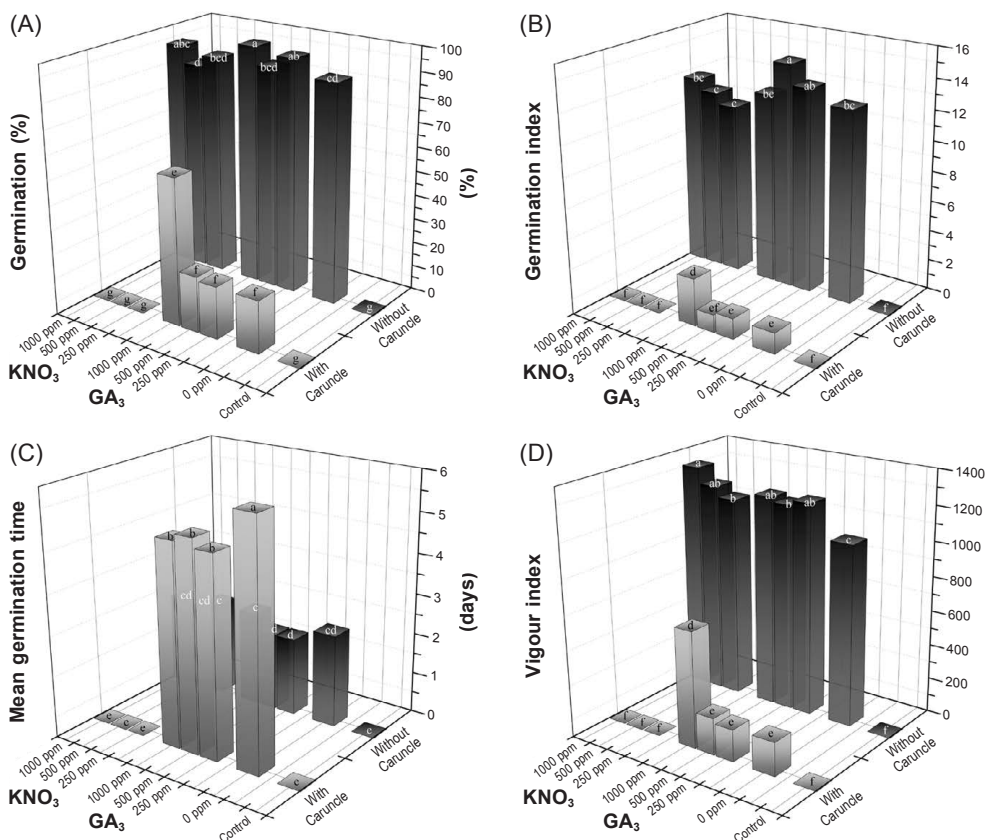


Figure 1. Changes in germination percentage (A), germination index (B), mean germination time (C) and vigour index (D) of castor seeds as affected by the presence of caruncle and different concentrations of GA<sub>3</sub> and KNO<sub>3</sub>. Columns within each graph with the same letter were not significantly different ( $P < 0.05$ ).

Approximately six times higher vigour index was obtained in caruncle-removed seeds compared to the control (table 1). While the mean vigour index in seeds treated with GA<sub>3</sub> was 593, it was 493 in seeds treated with KNO<sub>3</sub>. The highest vigour index was calculated in 1000 ppm GA<sub>3</sub> application in seeds with caruncle, but it was higher in 1000 ppm KNO<sub>3</sub> application in seeds without caruncle (figure 1).

Seedling growth parameters such as shoot length, root length, seedling fresh weight, dry matter and vigour index were significantly influenced by the presence of caruncle, priming agents, doses and their interactions. The seeds without caruncle produced longer seedlings than the seeds with caruncle. Also, GA<sub>3</sub> treatments induced root development (75.4 mm), while the seeds treated with KNO<sub>3</sub> gave longer shoots with 13.5 mm (figure 2). Hydration led to improved root elongation of castor seeds, but the highest levels of GA<sub>3</sub> and KNO<sub>3</sub> enhanced shoot elongation. The longest roots were measured at 500 and

Table 1. Germination characteristics of wild castor bean seeds with or without caruncle primed by different concentrations of GA<sub>3</sub> and KNO<sub>3</sub>.

Factors	Germination (%)	Mean germination time (day)	Germination index	Vigour index
Existence of caruncle (A)				
With caruncle	14.7 <sup>b</sup>	2.68 <sup>a</sup>	0.8 <sup>b</sup>	145 <sup>b†</sup>
Without caruncle	73.5 <sup>a</sup>	1.73 <sup>b</sup>	10.2 <sup>a</sup>	941 <sup>a</sup>
Priming agent (B)				
GA <sub>3</sub>	50.0 <sup>a</sup>	2.93 <sup>a</sup>	6.1 <sup>a</sup>	593 <sup>a</sup>
KNO <sub>3</sub>	38.1 <sup>b</sup>	1.48 <sup>b</sup>	5.0 <sup>b</sup>	493 <sup>b</sup>
Doses (C)				
Control	0.0 <sup>d</sup>	- <sup>c</sup>	- <sup>b</sup>	- <sup>c</sup>
0 ppm (Hydration)	55.5 <sup>b</sup>	4.10 <sup>a</sup>	7.0 <sup>a</sup>	617 <sup>b</sup>
250 ppm	52.0 <sup>c</sup>	2.28 <sup>b</sup>	6.6 <sup>a</sup>	642 <sup>b</sup>
500 ppm	50.0 <sup>c</sup>	2.27 <sup>b</sup>	7.0 <sup>a</sup>	656 <sup>b</sup>
1000 ppm	62.8 <sup>a</sup>	2.37 <sup>b</sup>	7.1 <sup>a</sup>	799 <sup>a</sup>
Analysis of variance				
A	**	**	**	**
B	**	**	**	**
A×B	**	**	ns	**
C	**	**	**	**
A×C	**	**	**	**
B×C	**	**	**	**
A×B×C	**	**	**	**

† Means followed by the same letter(s) in each factor are not significantly different at  $P < 0.05$ . Bracket (-) shows no data due to insufficient germination. \*\* = Significant at  $P < 0.01$ ; ns = not significant.

1000 ppm doses of both priming agents, while the longest shoots were obtained from the caruncle-removed seeds treated with KNO<sub>3</sub> (figures 3 and 4). Depending on root and shoot development, seedling fresh weight was enhanced by caruncle removal (488 mg seedling<sup>-1</sup>) and GA<sub>3</sub> (436 mg seedling<sup>-1</sup>). Hydrated seeds produced heavier seedling fresh weight than the application of GA<sub>3</sub> and KNO<sub>3</sub> (table 2 and figure 2).

Dry matter was considerably changed by caruncle removal, priming agent, doses, and all combinations of their interactions (table 2). It was higher in seedlings produced by seeds primed with KNO<sub>3</sub> (11.3%) than GA<sub>3</sub> (9.7%). The highest dry matter was obtained from hydrated seeds with caruncle and increasing GA<sub>3</sub> levels caused a decrease in dry matter (figure 2). No measurement of dry matter from the seeds treated with KNO<sub>3</sub> would be recorded because of an insufficient number of seedlings developed; consequently, a limited effect of KNO<sub>3</sub> on the dry matter was observed (figure 2).

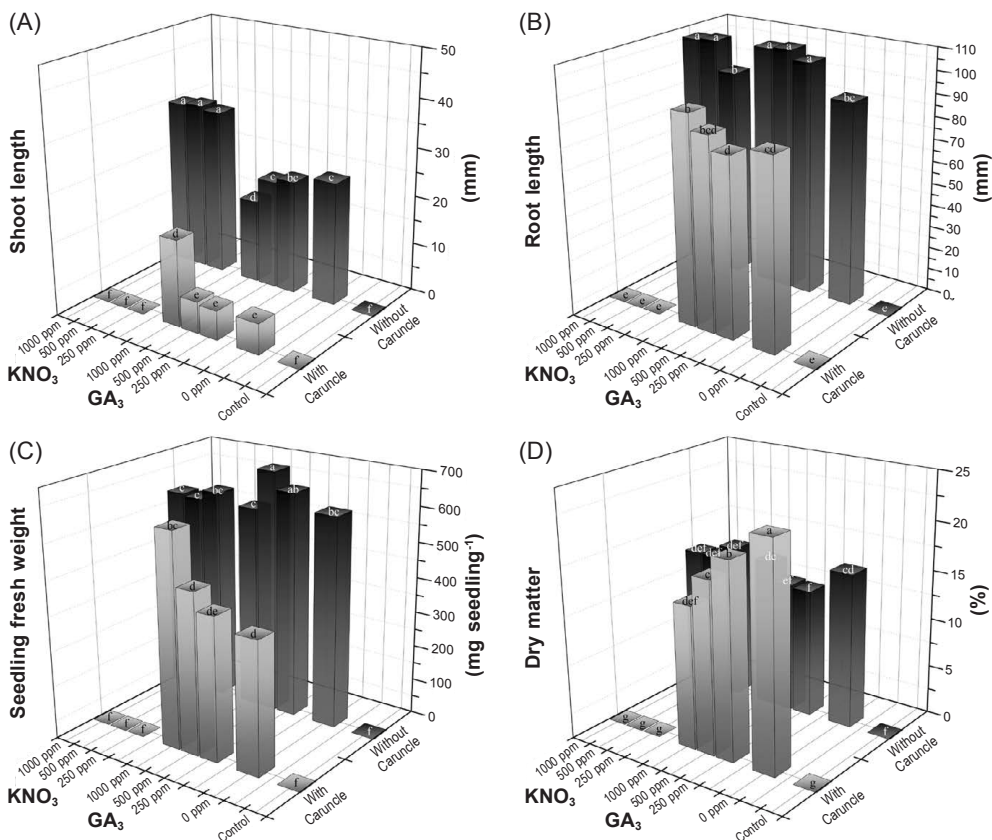


Figure 2. Changes in shoot length (A), root length (B), seedling fresh weight (C) and dry matter (D) of castor seeds as affected by the presence of caruncle and different concentrations of GA<sub>3</sub> and KNO<sub>3</sub>. Letters on each bar show significance level of means at  $P < 0.05$ .

## Discussion

The seeds of wild castor bean did not germinate under optimum conditions while removing caruncle enhanced germination. Priming promoted germination of the seeds with or without caruncle. The effect of priming was more prominent in seeds without caruncle than in seeds with caruncle (table 1). Similar results were reported by Bianchini and Pacini (1996) and Lagoa and Pereira (1987): an increase in the germination of castor seeds by removing the caruncle; and Rajesh and Amit (2010) found an increase in germination from 72 to 86.5% when the seeds were immersed in distilled water for 24 hours. Removing the caruncle can induce germination ability by permitting water and nutrients to enter the seed (Junior *et al.*, 2010) or by decreasing pathogen attack that begins in the fleshy portion of the seed (Guimarães and Cogni, 2002; Martins *et al.*, 2006).

Table 2. Seedling growth of wild castor bean seeds with or without caruncle primed by different concentrations of GA<sub>3</sub> and KNO<sub>3</sub>.

Factors	Root length (mm)	Shoot length (mm)	Seedling fresh weight (mg seedling <sup>-1</sup> )	Dry matter (%)
Existence of caruncle (A)				
With caruncle	42.8 <sup>b</sup>	4.3 <sup>b</sup>	223 <sup>b</sup>	13.2 <sup>a†</sup>
Without caruncle	80.8 <sup>a</sup>	21.8 <sup>a</sup>	488 <sup>a</sup>	8.2 <sup>b</sup>
Priming agent (B)				
GA <sub>3</sub>	75.4 <sup>a</sup>	12.5 <sup>b</sup>	436 <sup>a</sup>	9.7 <sup>b</sup>
KNO <sub>3</sub>	48.1 <sup>b</sup>	13.5 <sup>a</sup>	274 <sup>b</sup>	11.3 <sup>a</sup>
Doses (C)				
Control	<sup>-d</sup>	<sup>-c</sup>	<sup>-d</sup>	<sup>-c</sup>
0 ppm (Hydration)	87.5 <sup>a</sup>	15.8 <sup>b</sup>	489 <sup>a</sup>	19.3 <sup>a</sup>
250 ppm	69.4 <sup>c</sup>	15.9 <sup>b</sup>	414 <sup>b</sup>	11.7 <sup>b</sup>
500 ppm	75.3 <sup>b</sup>	16.0 <sup>b</sup>	432 <sup>b</sup>	11.3 <sup>b</sup>
1000 ppm	76.6 <sup>b</sup>	17.4 <sup>a</sup>	442 <sup>b</sup>	11.0 <sup>b</sup>
Analysis of Variance				
A	**	**	**	**
B	**	**	**	**
A×B	**	**	**	**
C	**	**	**	**
A×C	**	*	**	**
B×C	**	**	**	**
A×B×C	**	**	**	**

† Means followed by the same letter(s) in each factor are not significantly different at  $P < 0.05$ . Bracket (-) shows no data due to insufficient seedling growth. \*, \*\* = significant at  $P < 0.05$  and  $P < 0.01$ , respectively.

Mean germination time was shortened when the caruncle was removed from the seeds and primed with KNO<sub>3</sub> (table 1). Similarly, accelerated germination was reported by Mendes *et al.* (2009), by scraping the seeds without caruncle with sandpaper. Our results agree with the findings of Aghilian *et al.* (2014), who reported that GA<sub>3</sub> and KNO<sub>3</sub> had beneficial effects on breaking secondary dormancy by regulating hormonal balance and reducing germination inhibitor substances.

Seedling growth of wild castor seeds with or without caruncle was stimulated by GA<sub>3</sub> (table 2). This aligns with previous research findings of Zhou *et al.* (2014). Jadhav *et al.* (2022) also demonstrated a heavier seedling dry weight from chia seeds primed with GA<sub>3</sub> than hydropriming, KNO<sub>3</sub>- or salicylic acid-treatment. However, Thiruppathi *et al.* (2018) presented more promising results in seeds primed with 3% KCl and KH<sub>2</sub>PO<sub>4</sub>, and 2% ZnSO<sub>4</sub> for improving seedling development. Msaakpa *et al.* (2013) reported both a slower seedling development in the seeds with a caruncle and a heavier seedling weight in

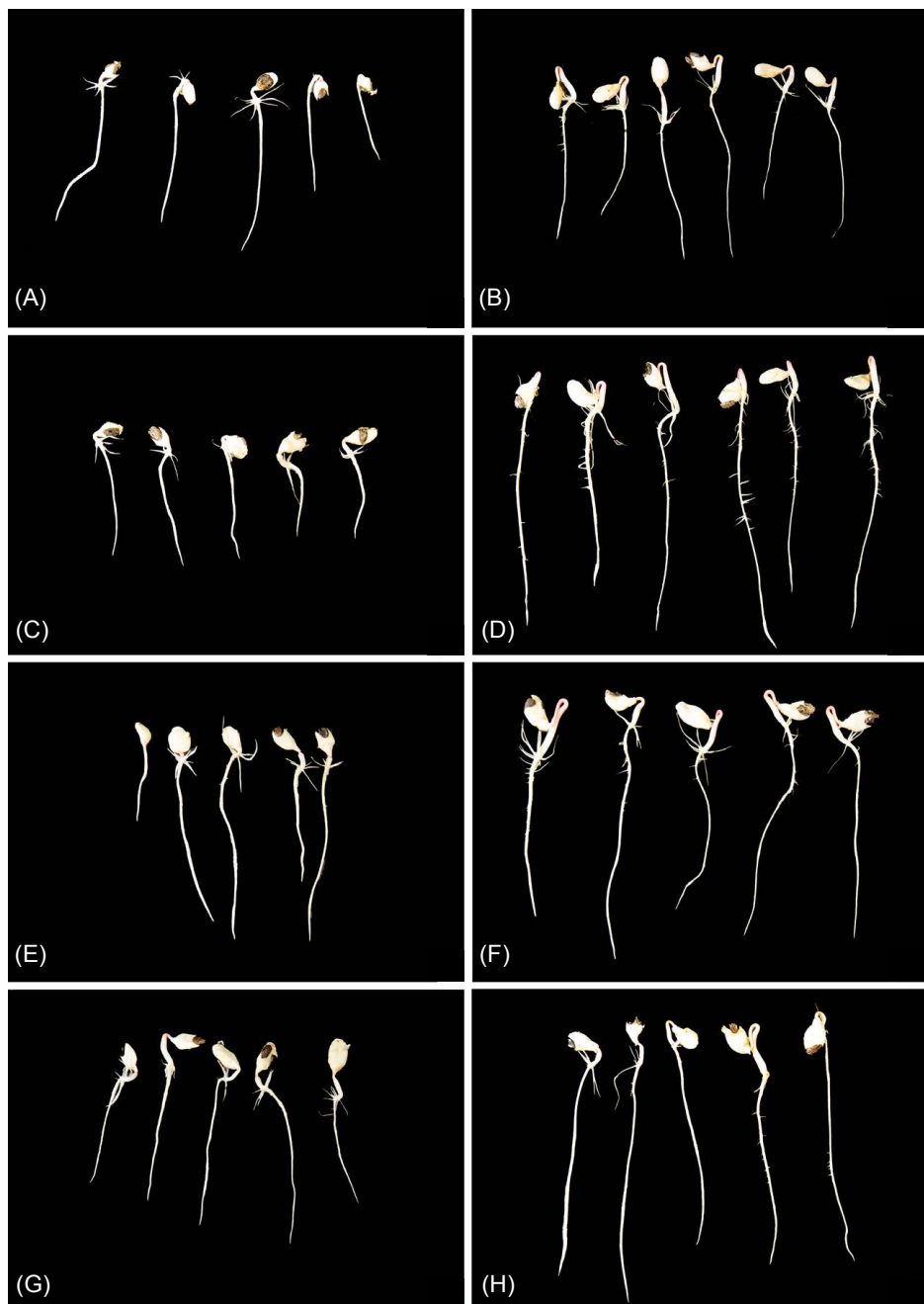


Figure 3. Seedling growth of wild castor seeds with caruncle primed with hydration (A), 250 ppm  $GA_3$  (C), 500 ppm  $GA_3$  (E), 1000 ppm  $GA_3$  (G), and the seeds without caruncle primed with hydration (B), 250 ppm  $GA_3$  (D), 500 ppm  $GA_3$  (F), and 1000 ppm  $GA_3$  (H).

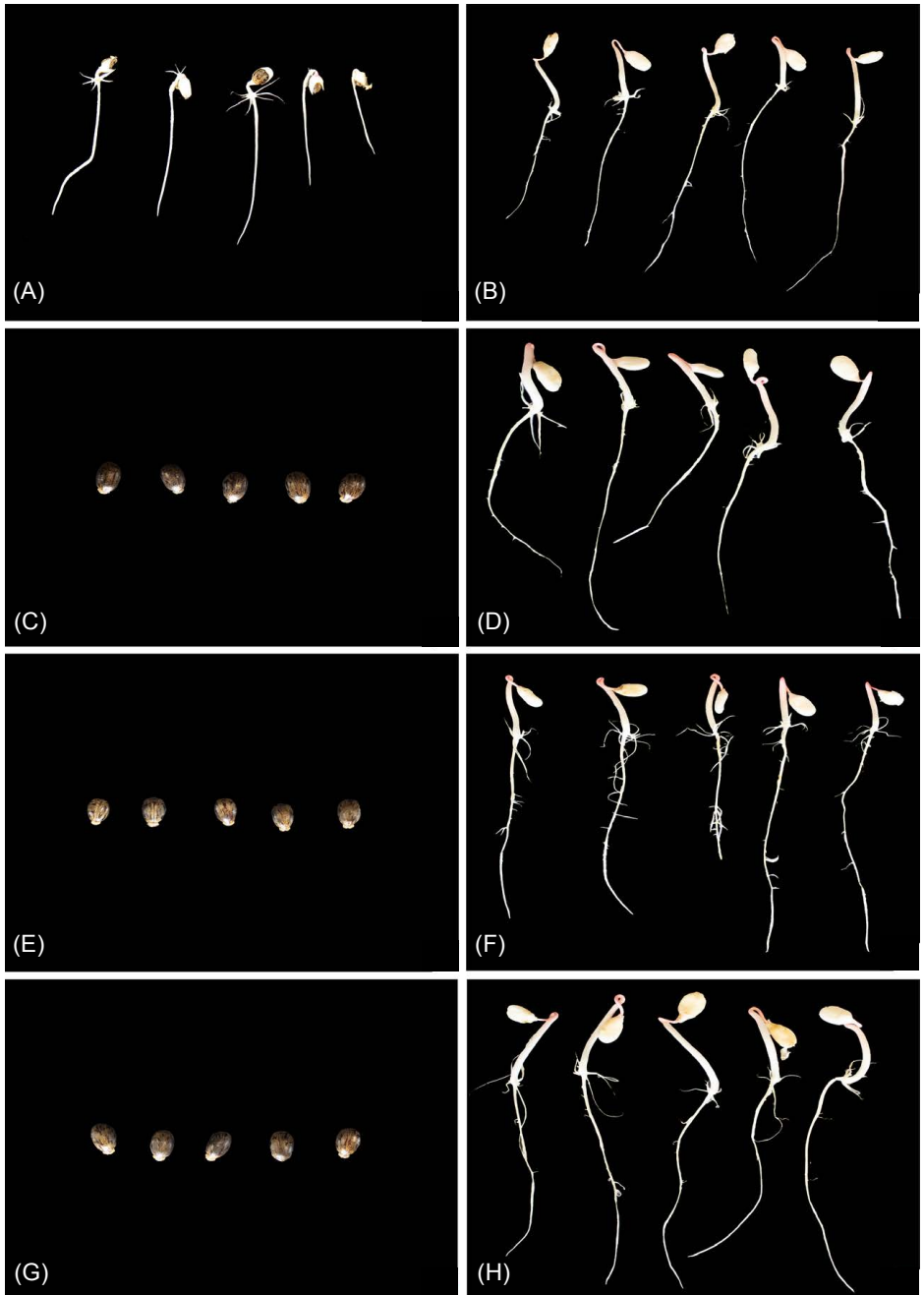


Figure 4. Seedling growth of wild castor seeds with caruncle primed with hydration (A), 250 ppm KNO<sub>3</sub> (C), 500 ppm KNO<sub>3</sub> (E), 1000 ppm KNO<sub>3</sub> (G), and the seeds without caruncle primed with hydration (B), 250 ppm KNO<sub>3</sub> (D), 500 ppm KNO<sub>3</sub> (F), and 1000 ppm KNO<sub>3</sub> (H).

seeds scarified compared with non-scarified seeds. Also, our results are similar to those of Bagale *et al.* (2022), who found improved stem elongation and dry matter accumulation in vegetable seedlings produced from seeds primed with GA<sub>3</sub>. Similarly, Jiao *et al.* (2019) announced that seed soaking with 250 ppm GA<sub>3</sub> promoted seedling growth of castor beans, and the seeds without caruncle given 500 ppm GA<sub>3</sub> produced the heaviest seedling fresh weight.

## Conclusion

In this study, the presence of the caruncle is not solely responsible for the germination failure in castor bean seeds. Nonetheless, caruncle removal was beneficial for the penetration of water into the seeds. The seeds without caruncle exhibited a higher germination percentage and index, along with a shorter mean time to germinate than the seeds with caruncle. Seed priming promoted germination of castor bean seeds, but their efficiency depended on caruncle removal. The GA<sub>3</sub> primed seeds without caruncle had the highest germination rate and produced vigorous seedling development. The results suggest that wild castor seeds without caruncle primed with 1000 ppm GA<sub>3</sub> provide new opportunities for cultivation and using native wild ecotypes in breeding programmes, because this treatment led to alleviation in seed dormancy and improvement in germination performance and seedling growth of wild castor bean. The higher doses of GA<sub>3</sub> should be tested for the seeds with caruncle of castor bean in further experiments.

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