



Effect of plant growth regulators and nitrogenous compounds on seed germination of pepper (*Capsicum frutescens* L)

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ABSTRACT: The seed germination of *Capsicum frutescens* L. was investigated through various methods, namely, washing and air dry storage treatments, applications of gibberelic acid (GA₃), Kinetin, potassium nitrite (KNO₂) and potassium nitrate (KNO₃) to improve germination of seeds in view of poor germination reported. The seeds were subjected to the following treatments namely washing to remove the slimy coverings; air-drying at various time-intervals; incubated in different concentrations of GA₃, Kinetin, KNO₂ and KNO₃. The treatments were subjected to light or dark germinations at 30°C and radicle emergence scored after 15 days. The various results showed that washing treatment, and light germination had a significant effect on the germination of *Capsicum frutescens* seeds. For air-drying treatment, germination increased from 0-48hrs and decreased after 72hrs, 48hrs air-drying duration gave the highest percentage germination. Application of GA₃, kinetin, KNO₂ and KNO₃ in low concentrations promoted the germination of freshly harvested *Capsicum frutescens* seeds. Generally the KNO₂ and KNO₃ gave the best germination results. The findings of this study have significant implications in the commercial production of *Capsicum frutescens* in view of its economic and medicinal importance. ©JASEM

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The genus *Capsicum frutescens* comprises more than 200 varieties, and the fruits vary widely in size, shape, flavour and sensory heat. Five main species are cited in literature: *Capsicum annum*, *Capsicum baccatum*, *Capsicum chinense*, *Capsicum frutescens* and *Capsicum pubescens*. Peppers from *Capsicum* species are native to the tropical and humid zones of Central and South America and belong to the *Solanaceae* family (Zimmer *et al.*, 2012). There are many local cultivars grown in West Africa. Nigeria alone has more than 200 selections of pepper (Idowu-Agida *et al.*, 2012).

The habits and habitats of *Capsicum frutescens* have been extensively described in various literatures. It is an annual or short-lived perennial herb with height of 1-4 feet; leaves are elliptical, dark green and smooth, and measures 2½ inches long and 1 inches wide; the flowers are typically conical or funnel form; the fruits are erect, ellipsoid conical to lanceoloid, 10-20mm long, 3-7mm in diameter; while the fruits range in colour from green when immature to purple, red, orange or yellow when ripe (Kirtikar and Basu, 1980; Menhichini *et al.*, 2009).

One of the problems that farmers face is the germination of pepper seed, that even under

favourable conditions, is slow and uneven, thus suggesting the existence of a certain type of dormancy in seeds of these species (Edwards and Sundstrom, 1987), which justifies the use of methods which help to accelerate the process of germination and uniform emergence in the field. Among the chemicals that are used for rapid and uniform seed germination are the plant growth regulators and biostimulants that control metabolism and the responses of seeds to the environment (Davies, 1994).

Capsicum frutescens is widely used as food flavouring agent, a colouring agent, an additive in livestock feed and pharmaceutical industries; excellent sources of various antioxidant compounds like flavonoids, carotenoids and vitamin C; fruits contain capsaicin which is used in modern medicine as a circulatory stimulant, pain reliever and an aerosol extract of capsaicin (pepper spray) are used by the police as non-lethal means of incapacitating a person (Boonkird *et al.*, 2009; Zhuang *et al.*, 2012; Oboth and Rocha, 2007).

It is believed that biostimulants, depending on the composition, concentration and proportion of substances, can increase plant growth, stimulating cell division, as well as increasing the absorption of water

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and nutrients for plants. The pre-soaking in plant growth regulators and biostimulants solutions can influence seed germination by accelerating the whole processes, which starts with the absorption of water, ending with the embryonic axis elongation (Bewley *et al.*, 2013). Studies performed in order to increase the germination of seeds of peppers (Fialho *et al.*, 2010), testing the effect of priming in seeds of yellow-long-pepper- (*Capsicum annuum* L.) and the use of various chemical treatments, including moistening of the substrate with gibberellic acid, aminocyclopropane 1-carboxylic acid, polyethylene glycol, KNO₃ and pre-cooling for rapid and uniform germination of seeds of true-cumari-pepper (*Capsicum baccatum* var. *Praetermissum* L.).

Reports of promotory effects in dormancy breaking and enhancement of seed germination of some of these treatments and chemical substances for other species have been noted (Hsiao, 1979a; 1979b; Onofeghara, 1981; Nzegbule and Mbakwe, 1999; Tambari and Aminu, 2015; Al-Menaie, 2010; Ramamoorthy, 2005; Irfan *et al.*, 2013; Ajiboye, 2010). The germination of *Capsicum frutescens* seeds, even under favourable conditions, is slow and disuniform and freshly sown seeds undergo rapid decay (Ado, 1987). These findings implied unavailability of the seeds in required quantity and at required time. Thus there is need to ensure continuous availability of this valuable plant species. Reports on the seed germination of *Capsicum frutescens* have been lacking or few in between. This study therefore intends to identify treatment methods to enhance germination of *Capsicum frutescens* in view of its economic, medicinal and agricultural potentials and uses.

MATERIALS AND METHODS

Source of Seeds: The matured fruits of *Capsicum frutescens* seeds were harvested from Agricultural Development Programme (ADP) Rumuodomaya, Rivers State. Fresh fruits which had not under gone any decay were collected and used for the experiment.

Viability Test: The Triphenyl tetrazolium chloride test (TTC) as developed by Lakon (1949) was used. The test is based on the understanding that living seed releases hydrogen molecules as a result of the action of dehydrogenase enzymes. The hydrogen molecules so released reduced the oxidized colourless form of TTC to formazan-red colour. Seeds were split longitudinally through the embryo and soaked in 5% solution of 2,3,5- Triphenyl tetrazolium chloride (TTC), then left for 2hours in the dark. The embryonic region was then examined for accumulation of formazan by noting the amount of red colour. The

embryonic sections of the seeds were coloured formazan red, indicating viability and were used for the experiment.

Treatment Methods: Washing: Germination of unwashed and washed seeds were examined to determine if the slimy coverings on the seeds impede germination. Unwashed and washed seeds were germinated as described under the germination conditions.

Dry storage at Room Condition: The seeds of *Capsicum frutescens* were stored at room condition for various time-intervals ranging from 0hours, 12hours, 24hours, 36hours, 48hours, 60hours and 72hours. After each storage time, seeds were subsequently germinated at required temperature. The objective of such treatment was to find out if germination increases or decreases with dry storage period. The seeds that germinated were counted on daily basis for 15 days.

Gibberellic Acid and Kinetin Treatments: The effect of gibberellic acid and kinetin on the germination of *Capsicum frutescens* was investigated. The different concentrations applied were 3.5 mg/l, 35mg/l and 346 mg/l for GA₃, and 2mg/l, 22mg/l and 215 mg/l for kinetin. For each of the treatment, a control experiment was always set up alongside.

KNO₃ and KNO₂ Treatment: The effects of potassium nitrate and potassium nitrite on the germination of *Capsicum frutescens* were also investigated. The different effects of concentrations, namely 101mg/l, 505mg/l and 1010mg/l of potassium nitrate (KNO₃) and 85mg/l, 425mg/l and 850mg/l of potassium nitrite (KNO₂) were examined.

Germination Procedures: For each treatment carried out, four replicates of 20 seeds per replicate were put in a 9cm plastic petri dishes, lined with 9cm Whatmann Filter paper, moistened with 3mls of distilled water or the required chemical solution and germinated at 30°C. Germination was monitored for a period of 15days and radicle emergence was scored as an index of germination.

Statistical Data Evaluation: All data collected were subjected to statistical analysis such as Analysis of variance (ANOVA) and standard error means. New Duncan Multiple Range Test (NDMRT) was employed to separate means.

RESULTS AND DISCUSSION

Washing Treatment: The percentage germination for washed and unwashed seeds was 35% and 16%

respectively after 8 days of incubation in the dark. At the end of the experiment i.e. 15 days of incubation, percentage germination increased to 58% for washed and 52% for unwashed (Fig. 1). The rate of percentage germination was enhanced in the washed seeds over the unwashed (control). The unwashed seeds may have some substances that may inhibit the percentage germination. Light promoted the germination of *Capsicum frutescens* seed in both washed and unwashed treatments. The rate of percentage germination for washed seeds is significant over the

control (unwashed) at $P = 0.05$. Many seeds require light for germination to occur more rapidly e.g. seeds of *Betula pubescens* and *Pinus sylvestris* which germinates more rapidly if exposed to light. Seeds sown on the surface of the soil or lightly covered with soil germinated more rapidly (4 days) than those kept in the dark or planted in the deep (8 days), (Jeffrey, 2005). The promotion of germination might be phytochrome controlled, that is red light promotes while far red light inhibits

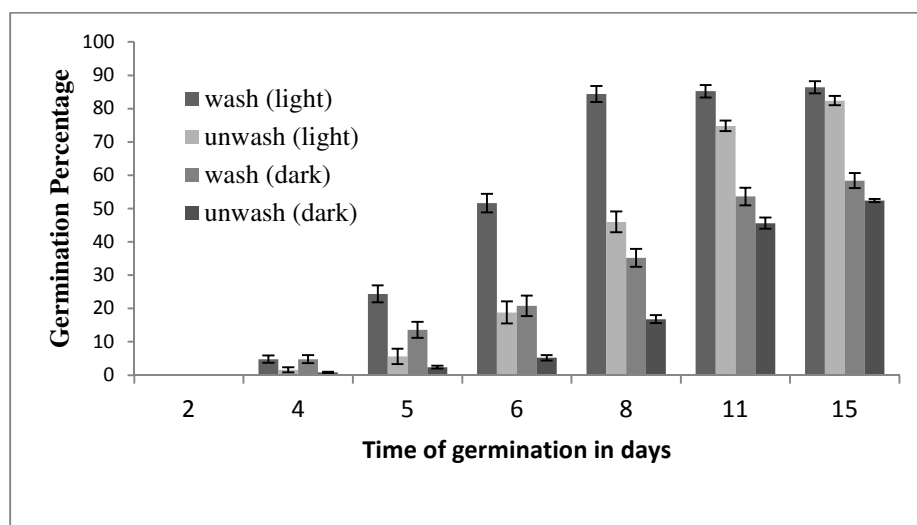


Fig.1: Effect of Light illumination and darkness on the germination of washed and unwashed seeds of *C. frutescens* germinated at 30°C

Dry Storage Treatment: The result of dry storage at room temperature (30°C) shows that the percentage germination gradually increases from 0hr to 48hrs and then gradual decreases after 72hrs air-dry storage (Fig. 2). The best time for drying was 48hrs, which gave the maximum percentage germination. The maximum percentage germination (48hrs) was not significant over the control at $P = 0.05$. Also, drying the seeds at different intervals did not significantly increase the percentage germination.

Ecologically, the effect of high temperature in the soil might act to produce drying in the soil, and may impose or release dormancy. In the laboratory, this

process is stimulated by keeping dry seeds at elevated temperatures in the range of 35°C – 50°C and is a form of “after-ripening”. It is regarded as any change which occurs in the seeds during storage as a result of which germination is improved (Evenari, 1965), however, in some cases, long storage drying could also impose dormancy e.g. in lettuce seed (Natile, 1945), decreased germination in *Traculia africana* after two weeks of storage. (Dolor, 2013). In this study, storage up to 48hrs enhanced germination. The resultant effect of “after-ripening” process is to widen the conditions under which dormancy can be broken and germination expressed.

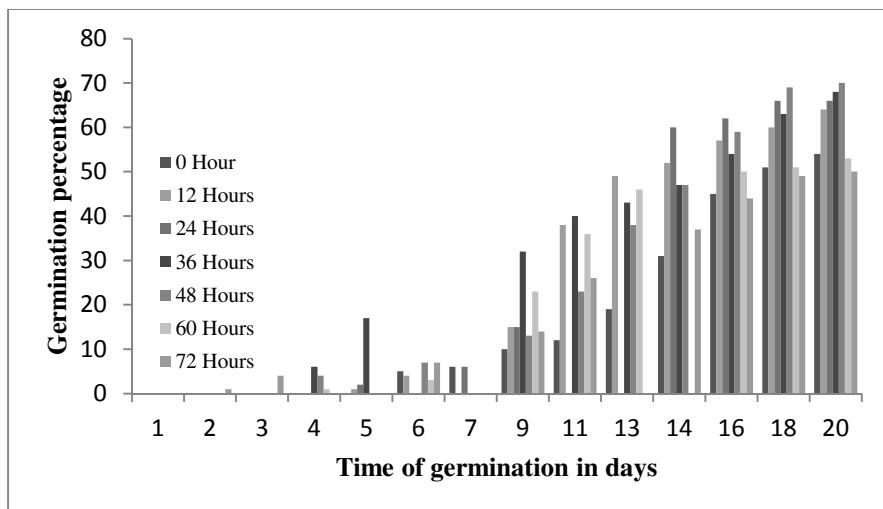


Fig. 2: The Mean percentage of seeds unwashed and kept dry at various time intervals (hrs) germinated at 30°C

GA₃ Treatment: Treatments with the 3 different concentrations of Gibberellic acid (GA₃) increased germination percentage over the control. 3.5mg/l, 35mg/l, and 346mg/l treatments gave germination percentages of 80%, 81.25% and 87.5% respectively, while the control gave 60% (Fig. 3). The percentage germination was not significant when compared to the 346mg/l treatment and the control at P=0.05. These results agreed with those of Mohammed (2007) in bean and Jamil and Rha (2007) in sugar beet. The stimulatory effect of gibberellic acid on germination percentage and seedling growth has been also reported in wheat (Akman, 2009). In fact, exogenous gibberellic acid causes an increase in germination and seedling growth by enhancing the availability of endogenous gibberellic acid (Kauer *et al.*, 1998). This could be explained by the fact that GA₃ may reduce the abscisic acid level in seeds through the activation

of their catabolism enzymes or by blocking the biosynthesis pathway (Toyomasu *et al.*, 1994). It has been reported by other researchers that Gibberellic acid (GA₃) is successful in ending dormancy in numerous species of seeds and also in accelerating the germination of non-dormant seeds. Effective concentrations generally lie within the range 0.10 – 10mM (Joshi, 1979).

Gibberellins have also been demonstrated in the control of the production of hydrolytic enzymes that ultimately lead to hydrolysis of seed food reserves and promoting germination (Paleg, 1965; Radley, 1967). In the endosperm of castor bean (*Ricinus communis*) seedlings, GA₃ stimulated the activities of at least 16 enzymes involved in gluconeogenesis, and the process of seed germination (Gonzalez and Delsol, 1981).

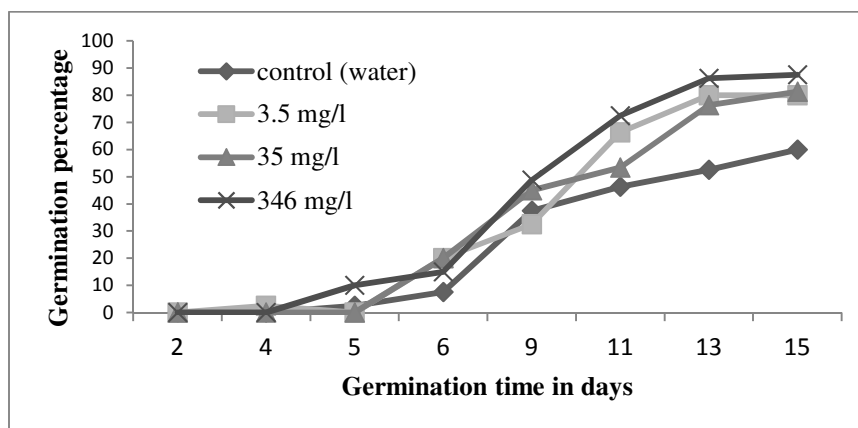


Fig.3: Effects of 3.5mg/l, 35mg/l, and 346 mg/l concentrations of Gibberellic acid on *C. frutescens* seeds germinated at 30°C

Kinetin Treatment: The results revealed the physiological response of *Capsicum frutescens* to kinetin at different concentrations. 2mg/l, 22mg/l and 215mg/l treatments gave germination percentage of 78.8%, 80% and 90%, respectively, while the control gave 55% (Fig.4). 215mg/l gave the highest percentage of 90%. The percentage germination were not significant when compared to the 215mg/l treatment and the control at $P=0.5$. The trend of germination promotion was in accordance with those

reported for *Arachis hypogea* (Ketrang and Morgan, 1971), *Cajanus cajan* (Mehta, 1978), and *Zea mays* (Bhatnagar and Rastogi, 1980). The mechanism by which kinetin promotes germination is not well understood. In *Phaseolus vulgaris* seeds incubated for four days, cytokinin released by the embryonic axis of seeds regulate the activity of a number of hydrolyzing enzymes which occur in the cotyledon (Van Staden *et al.*, 1982., thus promoting germination

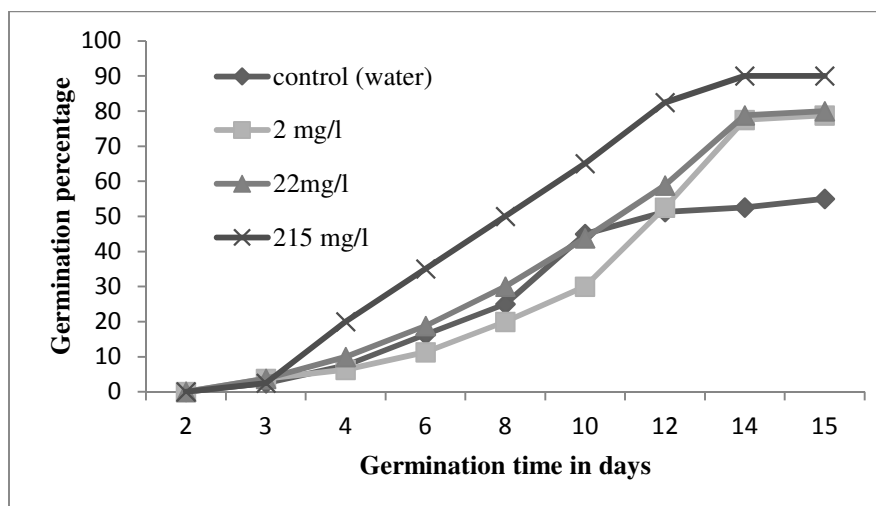


Fig.4: Effects of 2mg/l, 22mg/l and 215mg/l concentrations of kinetin on *C. frutescens* seeds germinated at 30°C

KNO₂ Treatment: The mean percentage germination of seeds treated with 85mg/l KNO₂ improved germination percentage over the control. 85mg/l KNO₂ gave the highest percentage germination of 81% while the control was 60% (Fig. 5). However, as the concentration of KNO₂ increases, the germination percentage decreases 425mg/l and 850mg/l gave percentage germination of 55% and 53% respectively (Fig. 5). There was no significant difference between the control and 85mg/l treatments even at 5% level of probability.

KNO₃ Treatment: The 101mg/l, 505mg/l and 1010mg/l treatments with KNO₃ gave germination percentages of 84%, 79%, and 61% respectively while the control was 58% (Fig. 6). 101mg/l treatment gave the highest percentage germination.

KNO₃ stimulates germination at low concentrations, but inhibits at higher concentrations. The percentage germination was significant when compared to the 101mg/l treatment and the control at $P=0.5$. These results seem to agree with the postulation of Mayer and Poljakoff – Mayber (1989) that nitrogenous

compounds such as nitrate and nitrite stimulate the germination of many seeds. Potassium nitrate and nitrite solution has long been known as a suitable chemical approach for promoting germination in various plant species and generally as a priming agent in germination media (Argerich and Bradford, 1989; Bush *et al.*, 2000; Madakadze *et al.*, 1993; McDonald, 2000; Tambari and Aminu, 2015; Al-Menaie, 2010; Ramamoorthy, 2005; Irfan *et al.*, 2013; Ajiboye, 2010; Faezeh and Vajihe, 2014; Hendricks and Taylorson, 1975; Roberts, 1973).

It has been speculated that these chemicals function to promote pentose phosphate (PP) pathway (Hendricks and Taylorson, 1975), and nitrate may functions by being reduced to nitrite or hydroxylamine and the hydroxylamine ions formed might be coupled to a respiratory chain which promotes germination through cyanide – resistant respiratory activity (alternative pathway). However, Eashi *et al.* (1979) rejected the hypothesis that nitrates and nitrites promoted germination by inhibiting catalase actions because KNO₂ and KNO₃ inhibited catalase activity but were ineffective in stimulating the germination of

unimbibed (dry) seeds of cocklebur. Juntilla and Nilsen (1980) noted that respiratory intensity is not correlated with germination of *Phalaris arundinacea*. It can be

concluded that the mechanism of action of KNO_2 and KNO_3 in promoting germination needs further investigation.

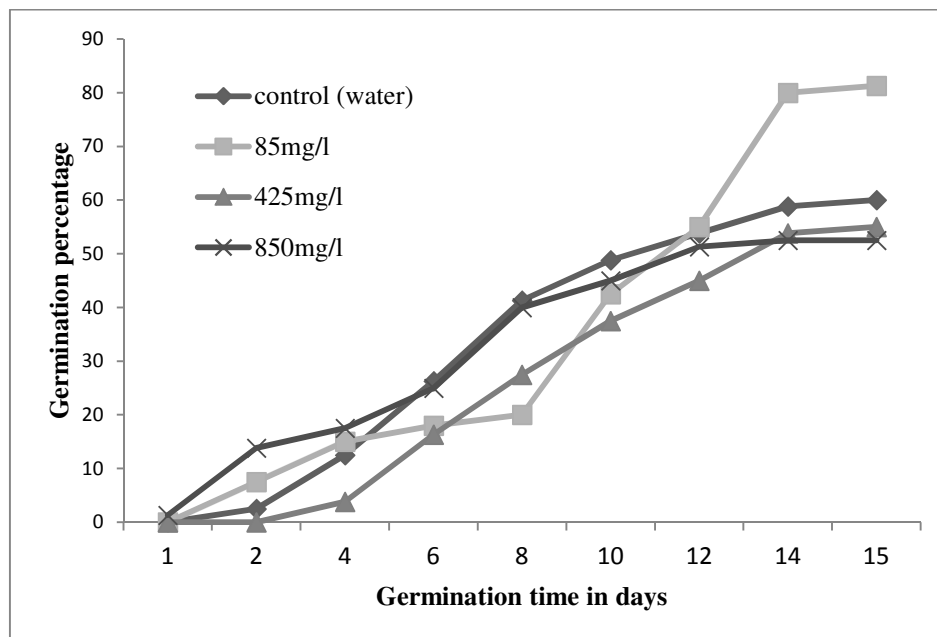


Fig.5: Germination response of seeds of *C. frutescens* to different concentrations of potassium nitrite germinated at 30°C

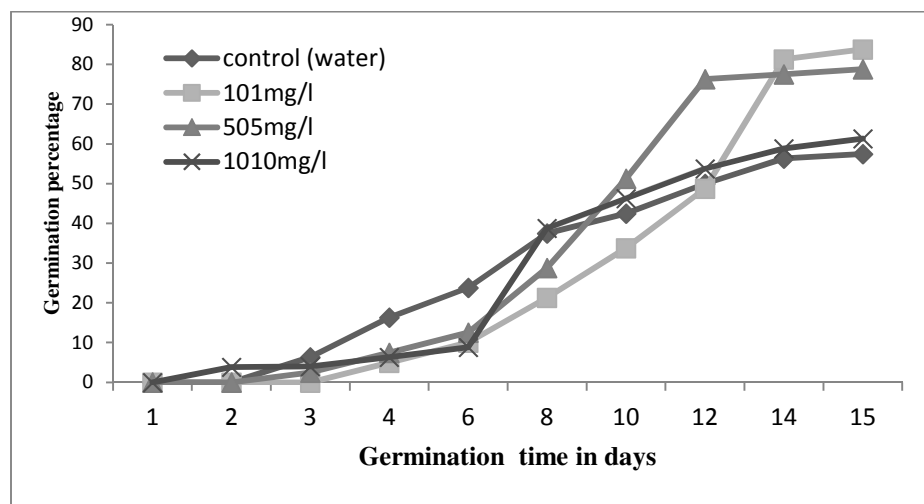


Fig.6: Germination response of *C. frutescens* seeds to different concentrations of Potassium nitrate germinated at 30°C

Conclusion/Recommendation: Seed germination of *Capsicum frutescens* can be enhanced by treatments such as washing of the seeds, dry storage at room temperature, incubations in GA_3 , kinetin, KNO_2 and KNO_3 . For optimum and increased production of the seeds of *C. frutescens*, it is recommended that seeds be grown in soils with low concentrations of KNO_2 and KNO_3 or soils enriched with these nitrogenous compounds in view of the challenges affecting the

seed germination and its huge economic, medicinal and agricultural potentials.

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