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Seed Dormancy Breaking in Nightshade (*Solanum trilobatum* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. Author MM carried out the experiment. Author VKM wrote the original draft. Author KS supervised the study, conducted the validation, and reviewed and edited the manuscript. Authors VSK and TK performed the analysis and interpretation of the results. Authors VKM and VSK also reviewed and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Nightshade (*Solanum trilobatum* L.) is one of the medicinal crops under Solanaceae family, often exhibits poor germination due to inherent seed dormancy. The present study was conducted to standardize the seed dormancy breaking treatments to improve the germination. The results clearly indicated that seeds treated with GA_3 @ 200 ppm for 6 h recorded maximum seed germination (84%) along with high speed of germination (5.5), seedling length (3.25 cm root length and 3.82 cm shoot length), dry matter production (4.94 mg seedlings^{-10}) and vigour index (584) when compared

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to treatments *viz.,* KNO_3 1.0% for 12 h (64%) and thiourea 0.5% for 6 h (50%), while the control recorded 38% of germination. The increase in duration of soaking with GA_3 beyond 6 h. significantly reduced the seed germination with concomitant changes in seedling quality parameters. Hence, it could be concluded that breaking the seed dormancy through exogenous application of GA_3, KNO_3 and thiourea confirmed the presence of physiological dormancy in nightshade seeds and it could be effectively broken through soaking the seeds in GA_3 @ 200 ppm for 6 h.

Keywords: Solanum trilobatum; dormancy; GA_3; KNO_3 and thiourea.

1. INTRODUCTION

Nightshade (*Solanum trilobatum* L.) is one of the medicinal plants belongs to the family Solanaceae. Traditionally, several medicinal plants such as aloe, kalmegh and Ocimum (tulsi) etc., have specific analgesic and antibacterial properties.The nightshade plants are reported to possess several immunomodulatory, anti-diabetic, anti-ulcerogenic and hepatoprotective properties. The leaf extracts are used to heal snake poison and improve male fertility (Kumar et al*.,* 2011). The entire plant decoction is used to treat acute and chronic bronchitis. According to cultivation, systematic cultivation of *Solanum trilobatum* L. is not practised anywhere, facing a range of difficulties in capitalising on their long-established advantages. Based on its ethnomedicinal importance, the plants have to be cultivated and conserved for the future generation. The mode of propagation of the crop *Solanum trilobatum* L. It is mainly propagated through seeds which facilitate easy conservation practices. There are many numbers of literatures which detailed about the medicinal uses of the crop, but the resources related to the seed quality characteristics are limited. On persual of several literatures, it was reported that fresh seeds of nightshade have very low germination potential due to the prevalence of seed dormancy (Alagumanian et al*.,* 2004), which might interfere the spread and distribution of plants. Seed dormancy is a biological mechanism that ensures seed germination at the appropriate time and under favorable conditions for the development and growth for the next generation. To ensure a uniform crop stand, it is highly essential to study the suitable seed dormancy breaking treatment to improve the seed germination.

2. MATERIALS AND METHODS

2.1 Experimental Sit and Treatment Details

The work has been carried out in the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore, in the year 2024-2025. The fully ripened berries were collected from ARS, Vaigaidam. The berries were graded based on their size and berries were surface sterilized with 2% sodium oxychloride (NaOCl) for 5 min and then treated with 80% ethanol for 5 min, followed by rinsing thrice with distilled water. Initial germination was low due to dormancy. The seeds were treated with different chemicals with different durations to break the seed dormancy. The treatment details are listed: T_0– Control, T_1 - GA_3 100 ppm, T_2 - GA_3 200 ppm, T_3 - KNO_3 0.5%, T_4 - KNO_3 1.0%, T_5 - Thiourea 0.5%, T_6 - Thiourea 1.0% and duration of soaking: D_0 - 6 hrs, D_1 -12 hrs, D_2 -18 hrs. After the treatment, the seeds are shade dried at room temperature and subjected to the germination test in roll towel method. The seed quality parameters were estimated as per the ISTA (2019) protocol, such as Speed of germination, germination %, seedling length (cm), vigour Index and dry matter Production (mg/10 seedlings) were recorded.

> Speed of germination = $X_1/Y1+X_2-X_1/Y2+\ldots+Xn- Xn\text{-}_1/Yn$

Here X_1, X_2… Xn are the frequency of germinated seeds on the first, second and final day. While Y_1, Y_2 … Yn are the days from sowing to first, second and up to last day.

> Germination (%) = Total number of seeds germinated/Total number of seeds sown × 100

Seedling length = Mean root length (cm)+ Mean shoot length (cm)

Vigour index= Germination (%) × Total seedling length (cm)

2.2 Statistical Analysis

The data were collected from the experiments has been carried out to analyse the statistical significance. The data were analysed by the

method which is described by (Gomez and Gomez, 1984). The percent values were converted into angular transformation (arc sine) values wherever necessary. The critical difference (CD) value was calculated using 5% probability level.

3. RESULTS AND DISCUSSION

Seed dormancy is considered a component of growth arrest. It is characterized by a partial metabolic arrest that initiates and terminates under the direction of endogenous hormones. A balance of growth promotor and inhibitor regulates the mechanism behind seed dormancy. During seed maturation, the activity of growth promoter tends to decrease therefore seed dormancy is induced (Amen et al., 1968). Seed dormancy is defined as inability of viable seeds to germinate under favourable conditions (Bewley, 1997). Seed dormancy is a mechanism that limits the seed germination and growth of embryo. The level of seed dormancy is commonly regulated by an interaction between the development of seed under different growth phases and environmental conditions experienced by the parent plant.

Many of the medicinal plants remains non-domesticated because of their wild growth in nature (Pallavi et al*.,* 2014). Due to non-domestication, systematic cultivation of many important medicinal plants is not practiced which results in extinction of several medicinal plants. For, effective cultivation of medicinal plants the seeds must be viable and non-dormant. Cultivation of dormant seeds results in poor germination and uneven crop stand. For uniform crops and the seeds must be non-dormant for cultivation. In this present study, an attempt was made to standardize the suitable dormancy breaking treatment for germination improvement in the nightshade.

The freshly extracted seed was subjected for germination without any pretreatment, which records only 38% of germination. The minimum rate of germination percentage indicates the presence of dormancy Freshly extracted seeds(dryseeds) were taken as control and there seeds were treated with GA_3, KNO_3 and thiourea for breaking seed dormancy to improve germination. The duration of the treatment was carried out for 6, 12 and 18h at ambient temperature. After the pretreatment, the seeds were subjected for germination to observe seedling quality parameters *viz*., germination, speed of germination, seedling length and vigour index of the seedlings.

The growth potential and survival of seeds is the prime indicator of germination percentage which is irrespective of factors that leads to loss of viability in seed (Abdalla and Roberts1969). Increasing the level of seed germination is the major objective in the successful seed production programme. One of the most important aspects of subjecting the seeds for germination test is mainly to determine the planting value of the seed for uniform crop establishment and to compare the test results between different seed lots.

In this present study, significant results were obtained among seed germination, the seeds treated with GA_3 200 ppm @ 6h. Improved the germination percentage (84%) when compared with control (38%) (Fig. 1). Seed treatment with GA_3 100 ppm@6h resulted 72% of germination which is next to GA_3 200ppm for 6hrs (Fig. 2) (Table 1). Similar results were reported by Jayamani (2020) in black cumin.

The increase in seed germination through GA_3 treatment is might be due to the enhanced stimulation of α-amylase synthesis and other hydrolyzing enzymes. The main role of hydrolyzing enzymes is the conversion of the complex starch molecules into simple sugars (Baskin et al*.,*1998, Finch-Savage and Leubner-Metzger 2006). Moreover, GA_3 accelerates the vegetative growth and it weakens the endosperm layer which limits embryo growth and it mobilizes the endosperm food reserves (Bareke, 2018).

Speed of germination have been used as an index of seed vigour and seedling growth rate. The time taken to the emergence of radicle from the seed coat is observed as speed of germination. It defines the ability of seed to produce seedlings with in a shorts pan of time under favourable conditions. Significant variations were observed in the speed of germination among different treatments and duration. Among various treatments, GA_3 200 ppm for 6h. significantly improved the speed of germination (5.5) when compared with control (2.7) (Table 1). The increase in speed of germination is mainly due to the breakdown of complex food materials in to simple sugars and release of energy into the growing tip of the radicle which triggers the speed of germination. The seeds which imbibe water but not germinated until the end of the germination test

period are termed as fresh ungerminated seeds. The seeds were able to germinate after an appropriate treatment. The minimum number of fresh ungerminated seeds were recorded in GA_3 200 ppm for 6h. (16%) when compared to control (54%) (Fig. 3) (Table 2).

Dry matter production and seedling length are the measure of the seed vigour index. It determines the physiological potential of germinating seeds (Heydecker et al., 1973). In the present investigation the seeds treated with GA_3 200 ppm for 6h. had significantly improved the seedling length (3.25 cm root length and 3.82 cm shoot length) when compared with control (2.19 cm root length and 3.08 cm shoot length) (Table 3) The seeds treated with GA_3 200 ppm for 6h. has recorded maximum dry matter production (4.94 mg seedlings^{-10}) when compared with control (2.66 mg seedlings^{-10}) (Table 4). The physiological phenomenon of seedling vigour is determined by reserve metabolites, enzymatic activity and growth regulators. The vigour factor determines the germinability of the seed which is the ultimate expression of seed quality (Heydecker, 1972).

Fig. 1. Effect of GA3, KNO_3 and thiourea on seed germination in nightshade

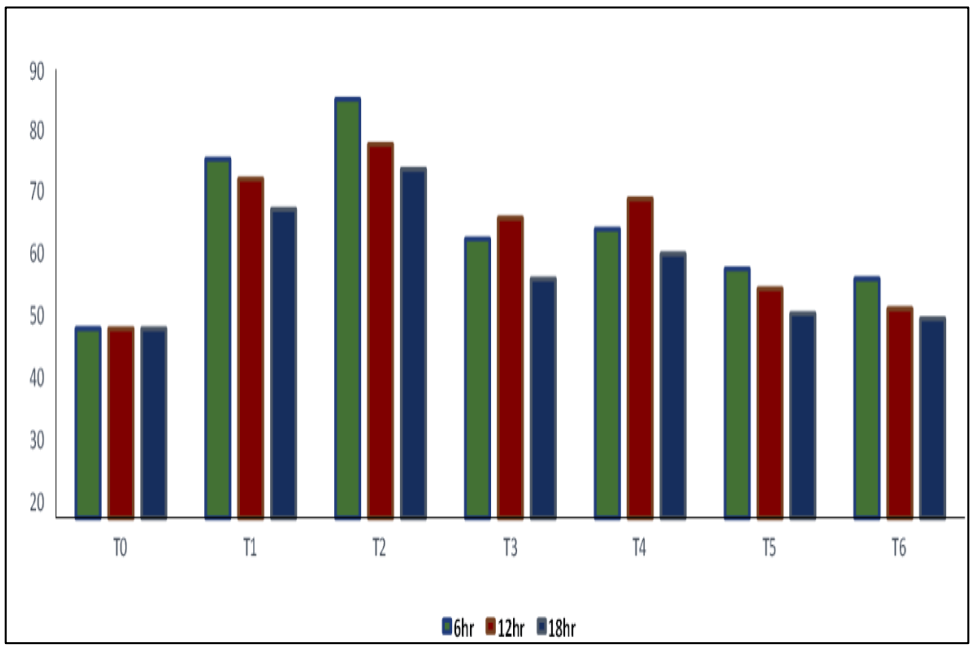

Fig. 2. Effect of different seed dormancy breaking treatment on seed germination (%) in nightshade

(T_0 – Control, T_1 - GA3 100 ppm, T_2 - GA3 200 ppm, T_3 - KNO_3 0.5%, T_4 - KNO_3 1.0%, T_5 - Thiourea 0.5%, T_6 - Thiourea 1.0%: D_0 - 6 hrs, D_1 -12 hrs, D_2 -18 hrs)

Table 1. Effect of GA3, KNO_3 and thiourea on seed germination (%) and speed of germination in nightshade (*Solanum trilobatum* L.)

Treatments(T)	Germination (%)				Speed of germination			
	Duration(D)			Mean	Duration(D)			Mean
	6 h	12 h	18h		6 h	12 h	18h	
T_0–Control	38(38.05)	38(38.05)	38(38.05)	38(38.05)	2.7	2.7	2.7	2.7
T_1-GA3100 ppm	72(58.05)	68(55.55)	62(51.94)	67(55.21)	4.4	4.2	4.0	4.2
T_2-GA3200 ppm	84(66.4)	75(60.00)	70(56.79)	76(61.12)	5.5	4.6	4.5	4.8
T_3-KNO30.5%	56(48.44)	60(50.76)	48(43.85)	55(47.74)	3.5	3.7	3.2	3.4
T_4-KNO31.0%	58(49.60)	64(53.13)	53(46.72)	58(49.82)	3.6	3.8	3.4	3.6
T_5–Thiourea0.5%	50(45.00)	46(42.70)	41(39.81)	46(42.55)	3.2	3.1	3.0	3.1
T_6-Thiourea1.0%	48(43.85)	42(40.39)	40(39.23)	43(41.15)	3.1	3.0	2.9	3.0
Mean	58(49.95)	56(48.69)	50(45.20)		3.7	3.5	3.3	
	T	**D**	**T×D**		**T**	**D**	**T×D**	
SEd	0.82	0.54	1.43		0.07	0.04	0.13	
CD(P=0.05)	1.65	1.08	2.87		0.15	0.09	0.26	

(Data in parentheses indicate arc-sine values)

Table 2. Effect of GA3, KNO3 and thiourea on fresh ungerminated seeds (FUG) in nightshade (*Solanum trilobatum* L.)

Treatments(T)	Duration(D)			Mean
	6 h	12 h	18h	
T_0–Control	54(47.29)	54(47.29)	54(47.29)	54(47.29)
T_1-GA3100 ppm	28(32.40)	30(33.42)	36(36.84)	31(34.00)
T_2-GA3200 ppm	16(24.50)	25(30.40)	28(32.25)	23(28.50)
T_3-KNO30.5%	44(41.44)	39(38.66)	48(43.45)	44(41.35)
T_4-KNO31.0%	42(40.10)	36(37.02)	44(41.08)	41(39.60)
T_5–Thiourea0.5%	44(41.55)	46(42.30)	48(43.51)	46(42.70)
T_6-Thiourea1.0%	46(42.70)	47(42.75)	48(43.31)	47(43.27)
Mean	39(38.43)	40(38.65)	43(41.31)	
	T	**D**	**T×D**	
Sed	0.50	0.33	0.87	
CD(P=0.05)	1.01	0.66	1.75	

(Data in parentheses indicate arc-sine values)

Table 3. Effect of GA3, KNO_3 and thiourea on root length and shoot length in nightshade (*Solanum trilobatum*L.)

Treatments(T)	Root length (cm)				Shoot length (cm)			
	Duration(D)			Mean	Duration(D)			Mean
	6 h	12 h	18h		6 h	12 h	18h	
T_0–Control	2.19	2.19	2.19	2.19	3.08	3.08	3.08	3.08
T_1-GA3100 ppm	2.75	2.36	2.20	2.43	3.54	3.43	3.18	3.35
T_2-GA3200 ppm	3.25	2.89	2.70	2.94	3.82	3.52	3.26	3.53
T_3-KNO30.5%	2.63	2.73	2.33	2.56	3.22	3.23	3.16	3.21
T_4-KNO31.0%	2.66	2.81	2.44	2.63	3.24	3.36	3.20	3.26
T_5–Thiourea0.5%	2.53	2.39	2.29	2.40	3.19	3.24	3.11	3.18
T_6-Thiourea1.0%	2.46	2.31	2.20	2.32	3.18	3.10	3.08	3.12
Mean	2.63	2.52	2.37		3.32	3.27	3.15	
	T	D	T×D		T	D	T×D	
SEd	0.04	0.03	0.08		0.06	0.04	0.11	
CD(P=0.05)	0.09	0.06	0.16		0.12	0.08	NS	

Table 4. Effect of GA3, KNO3 and thiourea on dry matter and vigour index in Nightshade (*Solanum trilobatum* L.)

Treatments(T)	Dry Matter (mg/10seedlings)				Vigour Index			
	Duration(D)			Mean	Duration(D)			Mean
	6 h	12 h	18h		6 h	12 h	18h	
T_0–Control	2.66	2.66	2.66	2.66	2.66	200	200	200
T_1-GA3100 ppm	4.69	4.18	3.84	4.23	4.69	440	394	334
T_2-GA3200 ppm	4.94	4.56	4.18	4.56	4.94	584	482	418
T_3-KNO30.5%	3.96	3.97	3.45	3.79	3.96	326	359	264
T_4-KNO31.0%	3.64	4.24	3.31	3.73	3.64	342	396	298
T_5–Thiourea0.5%	3.55	3.14	3.05	3.24	3.55	287	259	221
T_6-Thiourea1.0%	3.35	2.78	2.75	2.96	3.35	270	228	212
Mean	3.82	3.64	3.31		3.82	350	331	278
	T	D	T×D		T	D	T×D	
SEd	0.08	0.05	0.14		12.02	7.87	20.83	
CD(P=0.05)	0.16	0.10	0.28		24.03	15.73	41.62	

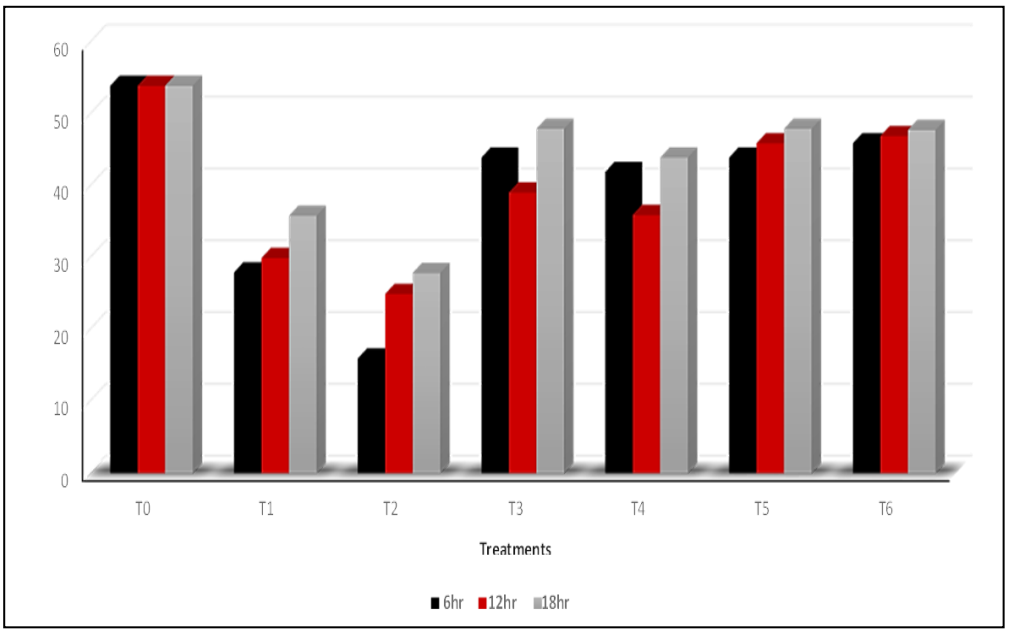

Fig. 3. Effect of different seed dormancy breaking treatment on fresh ungerminated seeds (%) in nightshade

(T_0 – Control, T_1 - GA3 100 ppm, T_2 - GA3 200 ppm, T_3 - KNO_3 0.5%, T_4 - $KNO_3$1.0%, T_5 - Thiourea 0.5%, T_6 - Thiourea 1.0%: D_0 - 6 hrs, D_1 -12 hrs, D_2 -18 hrs)

In the present study the maximum vigourindex was recorded in the seeds treated with GA3 200 ppm for 6h. (584) when compared with control (200) (Table 4). The improvement in vigour index is might be due to the fact, seed treatment with GA3 regulates high water absorption which results in increased cell wall elasticity and it leads to the formation of the effective root system which is responsible for an increase in vigour index.

The seeds treated with GA3 were significantly improved the germination percentage which ranged from 72% in 100 ppm to 84% ppm in 200 ppm, when the seeds were soaked for 6h. The increased duration of soaking in GA3 significantly reduced the germination per cent with concomitant changes in seed quality parameters such as speed of germination, seedling length, dry matter production and vigour index. These findings are in conformity with Keerthana (2018) in *Solanum nigrum*.

The seeds treated with KNO3 had also significantly improved the germination but not more than GA3 treated seeds. The seeds treated with KNO3 1% for 12 h. had improved the germination of 64 per cent and other seedling quality parameters such as speed of germination (3.8), root length (2.81 cm), shoot length (3.36 cm), dry matter production (4.24 mg/10 seedlings) and vigour index (396). These findings were correlated with Gupta et al. (2011), reported that the seeds of *Hippophae salicifolia* were treated with KNO3 @ 0.1% for 48h were significantly improved the seed germination and other quality parameters. The improvement in germination by KNO3 is mainly due to an increased level of oxygenby decreasing the supply ofoxygen to the citric acid cycle. More amount of oxygen was dissolved in water which makes the availability of more oxygen for the embryo which improves the rate of germination (Bewley and Black, 2012).The results are in conformity with (Roberts and Lockett,1978) noticed the improvement in germination percentage when the seeds of *Solanum nigrum* were treated with 0.2 % KNO3.

The seeds treated with thiourea showed slight improvement in seed germination and other seedling quality parameters. The results obtained from the seeds treated with thiourea 0.5% for 6 h. resulted in improved germination (50%), speed of germination (3.2), root length (2.53cm), shoot length (3.19cm), dry matter production (3.55mg seedlings^{-10}) and vigour index (287). The improvement in seed germination is mainly due to the process of acidification and loosening of cell wall and erodes the seed coat which

significantly improves the cell wall permeability for movement of water in the seeds (Ali et al., 2010). The germination and other quality parameters were decreased when the concentration and duration of soaking were increased. Similar results were reported by Pandey et al. (2000) in *Aconitum* sp and Gupta et al. (2011) in *Hippophae salicifolia*. However, the maximum seed germination was observed in seeds treated with GA_3 200 ppm for 6hrs soaking when compared with other growth regulators. The decrease in germination per cent were noted in GA_3 100 ppm for 18 hrs recorded 62 percent of germination and 48 percent of germination was observed in KNO_3 0.5% for 18hrs and the seeds treated with thiourea 1.0% for 18 hrs recorded only 40 per cent of seed germination. The results clearly depict that optimizing the concentration and duration of seed dormancy breaking treatment is important for each and every species.

4. CONCLUSION

The study concluded that, seeds treated with GA_3 200 ppm for 6 h has reported improvement in germination percentage (84 per cent) when compared with control (38 per cent) and also improved other seedling quality parameters. The reduction in germination percentage with concomitant changes in seedling quality parameters was recorded when the duration of soaking was increased. Hence, it is revealed that optimizing the concentration and duration of seed dormancy breaking treatment is critical for each and every species. The seed germination was also improved when it is treated with KNO_3 1% for 12 h. (64%) and thiourea 0.5% for 6 h. (50%). The results clearly indicated that breaking the seed dormancy through exogenous application of GA_3, KNO_3 and thiourea confirms the presence of physiological dormancy in nightshade (*Solanum trilobatum* L.) seeds.

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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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