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Effectof differentPre sowing treatments on seed germination of endangered medicinal plant Caesalpiniabonducella (L) Roxb.

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Abstract:*Caesalpiniabonducella(L.)Roxb.is an important Indian ethno medicinal plant belongs to the family Caesalpiniaceae. All parts of the plant including root, stem, bark, leaves, flower, pod, seed coat and kernel are used in many Ayurvedic preparations. The plant requires seven years for germination in nature due to hard seed coat. Hence this study deals with different treatments like mechanical scarification ,acid scarification, impact of effector substances like KNO_3, NaCl,$CdSO_4$, PEG and GA_3 on seed germination of nicker nut. Mechanically scarified seeds showed 40% germination. Acid scarification was carried out with con.HCl, con.H_2SO_4 and HNO_3. The treatments were given for 30 minutes, 60 minutes and 90 minutes in each acid. Con.H_2SO_4 for 60 minutes resulted in 80 % seed germination followed by 10%in Con. HNO_3 and 20% in Con. HCl. GA_3 50 ppm and 0.2%KNO_3 were also used and the germination rate were 40% and 10% respectively. Germination rate was 30% in 50mM NaCl. PEG and $CdSO_4$ were unsuitable for germination. In vitro seed germination was also studied with different phytohormones and agar. MS medium supplemented with 1mg/l NAA and 0.6 mg/l GA_3 resulted in 80% germination. When the concentration of the cytokinins, auxins and gibberellic acid were increased above 1mg/l there were no germination of seed.Seed germination rate was 90% in 2.5% plain agar medium. Radicle formation was noticed in 2% agar medium without shoot induction. The concentration of agar above 3% were not found to be effective for germination.*

Key Words:*Caesalpiniabonducella(L.)Roxb., germination rate, seed germination ,nicker nut.*

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I. Introduction:

Plants and human beings are interrelated to each other for centuries. It provides us food, fodder, shelter, ornaments and medicines. Traditional knowledge of medicine is passed on to generations after generations orally. Caesalpiniabonducella(L.)Roxb commonly known as nicker nut is an indigenous medicinal plant. The plant is reported as endangered in Malaysia and threatened in deciduous forest of western Ghats of India[12]. Nicker nut is used in all systems of medicines like Ayurveda, Unani, Siddha and homeopathy. The plant is reported to possess antimalarial activity and constitute an important ingredient of Ayush 64 used to cure malaria .Studies revealed that the the extract of Caesalpiniabonducella (L.)Roxb.Shows antibacterial, antioxidant[8], antidiabetic[10], antifilarial, antihelmintic, anti-inflammatory, antimicrobial, antifungal[7], antispasmodic, antidiarrhoeal, antiproliferative, antipsoriatic, antitumor[9], anxiolytic, anticonvulsant, antiamyoidogenic and antiviral activity.

Phytochemical analysis of the plant parts like leaf, root, stem, pod, seed coat and kernel revealed the presence of steroids, diterpenes, alkaloids, carbohydrates, tannins ,phenols, flavanoids and proteins. Bioactive Compounds like steroids, tri terpeness, alkaloids, carbohydrates and flavanoids are present in seed coat and kernel. The occurrence of most of the phytochemicals in seed of the plant justifies and support the use of seed as a major component of many pharmaceutical products. Caesalpiniabonducella(L.)Roxbpopulation is devastated for this reason and urbanization. Therefore present study was carried outto break the seed dormancy with different effector substances, growth hormones, acids and agar to conserve the plants of Caesalpiniabonducella(L.)Roxb.

II. Materials And Methods:

Seeds of Caesalpiniabonducella were collected from local Ayurvedic vendor. The seeds were washed thoroughly using labolene. Mechanical scarification was done with mortar and pestle. Cleaned seeds were immersed in concentrated HNO_3, $H_2SO_4$and HCl for 30 minutes, 60 minutes and 90 minutes respectively. After mechanical scarification and acid treatment the seeds were sown in soil. The germination rate was calculated by dividing the no. of seeds germinated to the total no. of seeds sown. The observations were taken at 5 days interval. Effector substances like KNO_3, $CdSO_4$, PEG, NaCl and GA_3 were used to pace the seed germination. KNO_3 5% and 10%,$CdSO_4$ 50M and 100M,PEG 5% and 10%,NaCl 50mM,75mM and 100mM and GA_3in

25ppm & 50ppm were also used for the germination . The germination rate and length of seedlings were recorded after 30,60 and 90 days. Control for all these experiments were seeds treated with distilled water. After treatment seed s were shown in garden soil.

In vitro seed germination was carried out using plain agar medium and MS medium supplemented with different phyto hormones. Agar medium was taken as control for this experiment. Observations were taken after 10,20 and 30 days and germination rate was calculated. MS medium was prepared using Standard protocol. The $_{P}H$ of the medium was adjusted to 5.8,temperature 25± 2^0c with 1000 lux light and 50%humidity. After 30 days the seedlings were transferred to pots containing garden soil mixed with sand(1:1).They were kept in the garden for another 30 days and then planted directly in the soil. Each experiment was carried out using 10 seeds and repeated thrice to find out the standard error. Statistical analysiswere carried out using ANOVA and p values were calculated to find out the level of significance of the experiment.

III. Result And Discussion:

Seeds were sorted out according tosize , color and shape .Healthy seeds were washed thoroughly and dried .when the seeds were sown directly the germination rate was zero. The seeds were mechanically scarified by making a small slit on the hard seed coat using moor and pestle.These seeds were sown in garden soil and the germination rate was 40%. However the seeds were taken 30 days for the emergence of radicle and the seedlings were established after 38 days. Acid scarification was done with Con.H_2SO_4,Con.HNO_3 and Con.HCl. The seeds were immersed in Con.H_2SO_4 for 30 min,60 min and 90 min. The germination rates were 20%,80% and 0% respectively.Radicle emergence was noticed after 14 days of sowing the seeds treated with Con.H_2SO_4 for 30 min. The seeds treated with Con.H_2SO_4 for 60 min. produced the radicle after 10 days of sowing in garden soil.Con.HNO_3 was also used for scarification of seeds and 10% germination was found in seeds treated for 30 min. and 60 min. Initiation of radicle was observed after 43 days of planting the seeds. The seeds were immersed in Con.HCl for 30min., 60 min. and 90 min. The germination rate was 10% and 20% for 30 min. and 60 min. respectively. Radicle emergence was found after 20 days of sowing the seeds.Beyond 60 min acid scarification with Con.H_2SO_4,Con.HCI and Con.HNO_3 were unsuitable for seed germination.

Effector substances like NaCl ,KNO_3 ,GA_3,$CdSO_4$ and PEG were used for breaking seed dormancy. The seeds were soaked in 50mM,75mMand 100mM NaClsoln for 24 hours and sown in the soil.The germination rates were 30% ,10% and 10% respectively. Radicles were grown above the soilafter 10 days of treatment.KNO_3 5% and 10% were used for seed soaking for 48 hours and the germination rates were 10% and 20% respectively. Radicle formation was initiated after 15 days of sowing and the seedlings were established in 20 days. The Con. Of GA_3 used for seed germination were 25ppm and 50ppm.The germination rate recorded were 40% for each concentration. Radicle initiation was noticed after 10 days. Even though $CdSO_4$ 50µM and 100µM were used for the study it was found to be insufficient for seed germination. Similarly PEG 5% and 10% were also found unsuitable for seed germination.

Seeds of Caesalpiniabonducella(L)RoxbSeedling after mechanical scarification.


Seedlings of acid Scarification in Con.H2SO4. Seedlings of acid scarificationinCon.HCl


Seedlings of acid Scarification in Con.HNO_3. Seedling in 10% KNO_3


Seedlings in 5% KNO_3. Seedlings in NaCl


Seedling in 25ppm GA_3. Seedling in50ppm GA_3


Table -1 :Effect of different treatments on in vivo seed germination of Caesalpiniabonducella(L)Roxb.

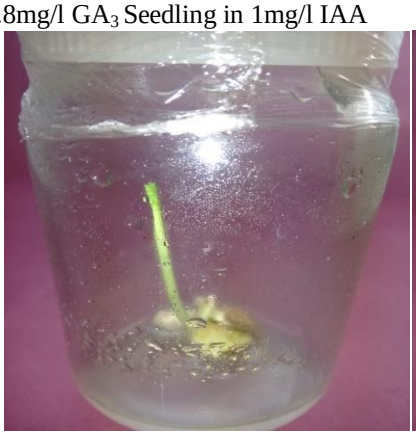
Treatment	Concentration	Time	Seedling length (cm ± SE)			Germination rate
NaCl	50mM	24 hrs.	16.96±0.023	26.53±0.067	52.39±0.089	30%
	75mM		09.54±0.102	15.02±0.066	22.14±0.091	10%
	100mM		08.37±0.197	12.07±0.026	14.96±0.043	10%
PEG	5%	24hrs.	-	-	-	-
	10%		-	-	-	-
$CdSO_4$	50µM	24hrs.	-	-	-	-
	100µM		-	-	-	-
KNO_3	5%	24hrs.	03.41±0.029	06.15±0.050	09.28±0.016	10%
	10%		02.69±0.066	04.92±0.072	07.60±0.067	10%
GA_3	25ppm	24hrs.	18.45±0.032	30.75±0.037	49.80±0.055	40%
	50ppm		18.99±0.060	32.26±0.016	49.66±0.066	40%
Con.H_2SO_4	98%	30min.	19.03±0.067	23.96±0.066	43.66±0.061	20%
	98%	60min	17.87±0.020	25.46±0.024	43.31±0.063	80%
	98%	90min.	-	-	-	-
Con.HCl	98%	30min.	12.44±0.062	25.69±0.030	49.84±0.058	10%
	98%	60min.	12.25±0.065	25.67±0.014	51.76±0.062	20%
	98%	90min.	-	-	-	-
Con.HNO_3	98%	30min.	17.75±0.006	32.31±0.059	53.77±0.019	10%
	98%	60min.	15.62±0.037	22.41±0.051	58.56±0.026	10%
	98%	90min.	-	-	-	-

In vitro technique was also used for seed germination. Mature seeds were taken for in vitro cultures. The seeds were decoated using mortar and pestle. The kernels were taken out and soaked in distilled water for 12 hours. These kernels were washed with running tap water for 30 minutes then treated with detergent. The kernels were again washed for 30 minutes under running tap water. Then kernels were exposed to UV for 30 minutes in laminar air flow chamber. The treated kernels were washed again thrice with distilled water. Then 1% $HgCl_2$ was used for the sterilization of explant and washed thrice with distilled water. These kernels were used for inoculation in MS medium supplemented with growth hormones like auxins, cytokinins and gibberellic acids. GA $_3$, NAA, IAA, IBA, BA and Kinetin 0.4 to 1mg/l were used to study in vitro seed germination. GA_3 0.4 to 1 mg/l produced radicle after 6 days of inoculation. The observations were taken at an intervel of 10,20 and 30 days. The length of the seedlings were measured after one month. GA $_3$ 0.8mg/l produced seedling of length 12.28±0.396 cm and the germination rate was 80%.MS medium supplemented with NAA 0.4 mg/l to 1mg/l were inoculated with seed kernel and the radicle formation was found after 5 days. Max. length of the seedlingin 1mg/l was 14.71± 0.041 cm and the germination rate was 80 %. Beyond 1mg/I NAA there was no germination. IAA 1mg/l in MS medium showed 30% germination and the length of the seedling was recorded as 12.42±0.228 cm .MS medium supplemented with IBA and 2,4- D produced seedlings with maximum length 16.27±0.020 cm in 0.8mg/l IBA and 16.52±0.469 cm in 1mg /l 2,4-D. The germination rate was recorded as 20% in 0.8mg/l IBA and 30% in 1mg/l 2,4-D. Kinetin 0.6 mg/l produced seedlings with max. length 16.49±0.370 cm and BA 0.4 mg/l with length 16.23± 0.106 cm. The germination rates were 20% in all these concentrations of Kinetin and BA (Table- 2). The agar medium was also used for seed germination. Agar 0.5% to 3% were studied for breaking seed dormancy. Agar 2.5% showed 90% germination and max. length of the seedling was 12.3±0.759 cm. Rhizogenesis was seen in 2% agar and shoot was not produced. Plantlets were planted in pots containing garden soil and sand (1:1).

Table-2 :Effect of different growth regulators on in vitro seed germination of Caesalpiniabonducella (L.)Roxb.

Treatment	Con. of phyto -hormones (mg/l)	Seedling length after 30 days (cm±SE)	Germination rate
GA3	0.4	11.25±0.104	20%
	0.6	11.81±0.590	20%
	0.8	12.28±0.396	80%
	1.0	9.30 ±0.352	20%
NAA	0.4	12.7 ±0.362	20%
	0.6	13.43±0.386	20%
	0.8	13.25±0.101	30%
	1.0	14.71±0.041	80%
IAA	0.4	10.51±0.286	20%
	0.6	11.36±0.490	20%
	0.8	12.13±0.759	20%
	1.0	12.42±0.228	30%
IBA	0.4	11.99±0.193	20%
	0.6	10.53±0.528	20%
	0.8	16.27±0.020	20%
	1.0	13.55±0.767	30%
2,4-D	0.4	12.48±0.270	20%
	0.6	13.70±0.715	20%
	0.8	13.91±0.132	20%
	1.0	16.52±0.469	30%
Kin.	0.4	15.14±0.159	20%
	0.6	16.49±0.370	20%
	0.8	12.92±0.211	20%
	1.0	14.13±0.171	20%
BA	0.4	16.23±0.106	20%
	0.6	14.47±0.104	20%
	0.8	13.34±0.102	20%
	1.0	13.71±0.230	20%

Seedling in 0.8mg/l GA_3 Seedling in 1mg/l IAA




Seedling in 1mg/l IBA (A)Rhizogenesis in 2% Agar medium


(B)Rhizogenesis in 2% agar mediumSeedling in 1mg/l NAA


Seedling in 1mg/l 2,4 –D. Seedling in 0.6mg/l Kin.

Seedling in 0.4mg/l BA

Seed germination and seed dormancy[5] are relishing topics of research among plant physiologists. The vital role of seeds in diet, therapy of many diseases, ethnic, ornamental uses and their integral part in traditional rituals leads to the extensive study ofseeds[6]. Medicinal plant seeds like Calotropis persia[1], Parkiabiglobosa(Jacq) Beth[3] and Melia azediarch[4] are characterized by their seed dormancy. Effect of different presowing treatments to induce seed germination were studied by many researchers. Seed germination of five medicinally important endemic plants in Western ghats were investigated by Rama Bhat. P in terms of seed emptiness and viability[14]. He observed 90% germination in these plants. According to Chang et al seed dormancy is a physiological factor affecting germination of seed. Size, color, shape and mass of the seeds are usually related with maturity and vigor[2]. Poor permeability of seed coat is a major barrier that restrains germination and that can be overcome by cutting, shaving, treating with low temperature and dipping it in chemical agents[15]. Caesalpiniabonducella(L) Roxb. has hard seed coat which inhibits germination[13]. Hence Nicker nut shows coat enhanced dormancy. So mechanical scarification, acid scarification and the use of different chemical reagents could break the dormancy[11]. All these were set the background to carry out seed germination studies in Caesalpiniabonducella(L)Roxb. Our studies reveals that both in vitro and in vivo methods can be adopted to enhance seed germination in nicker nut. Even though there were many reports for the medicinal importance and phytochemical studies of this plant, the seed germination studies are scanty. Thus the methods adopted for breaking the seed dormancy in our study can be useful to fasten seed germination and helps in protection of this endangered medicinal plant.

References:

[1]. AsgharFarajollahi, BarhamGholinijad and HamedJonaidiJafari. Effect of different treatments on seed germination improvement of Calotropispersica. Plant Science., 2014.

[2]. Chang, w., et all, Ying Yong Shing Tai Xue Bailey., Research advance in seed germination of desert woody plants, 2007,18, 436-444.

[3]. MAN Sambe, M Sagna and Moses.,Seed germination and in vitro plant regeneration of Parkiabiglobosa (Jacq.) Benth. African Journal of Biotech nology., 2010,9(21),3099-3108

[4]. MD Salim Azad, MdZedan-Al-Musa, MD. Abdul Matin., Effects of pre sowing treatments on seed germination of Meliaazedarach. Journal of forestry research., 2010,21(2),193-196

[5]. JD Bewley., Seed germination and dormancy. Plant cell., 1997,9(7),1055-1066.

[6]. A E Assogbadjo, R Glegrave, AF Azihon, Tina Kvndt. Jean. Ethnic differences in use value and use patterns of the threatened multipurpose scrambling shrub. (Caesalpiniabonducella L) in Benin. Journal of medicinal plants Research. 2011. 5(9), 1549-1557

[7]. ShrutiShukla, Pardee Mehta, Archana Mehta, Suresh Prasad Vyas, Vivek K Bajpai. Preliminary phytochemical and anti fungal screening of various organic extract of Caesalpiniabonducella seeds. Romanian Biotechnological Letters.2011. 16(4) , 6384-6389

[8]. Jayakrishnan, B.M.,NagarajaPerumal and Hashi K.M. In vitro antioxidant studies and phytochemical screening on the seeds of C.bonduc.Europian journal of experimental Biology. 2014.4(4),47-51.
[9]. OdeliaSawant, Girish N Zambre, Arulmozhis and Deepali A Bansode. Effect of Caesalpiniabonducella extract in pylori ligation induced ulcers in wistar rats. Derpharmacia Letter, 2016,8(4),191-197.
[10]. Mohammed Ahmad, DeepaliShaminJairapuri, Md. ShamimAkhtar, Moon Jain and Manju Sharma. Protective effect of Caesalpiniabonducella (Linn.) seed kernel extract in Streptozotocin induced hyperglycemia and oxidative damage in winstar rats. International journal of pharmaceutical sciences and Research, 2017, 2320-5148.
[11]. MR Khan. Seed dormancy in Caesalpiniabonducella (L) Roxb. Weekly Science Research Journal. 2014.1(46),2321-7871.
[12]. Jose AR Netopia, Vanessa S Stain Dra and Rosy IMA Ribeiro. Break of dormancy and evaluation of germination rate of the Cerrado medicinal plant :Eriosemapycnanthum. J. BiotechnolBiomater, 2017, 7(6), 64.
[13]. YashwantRai. Germination and growth status of endangered medicinal plant Caesalpiniabonduc (Linn.) Roxb. in Meerut (U. P.) India. International journal of innovation and scientific research. 2014. 6,50-55.
[14]. Rama Bhat P. Seed germination studies on some medicinally important endemic plants of the Western Ghats of Karnataka, India. Journal of global biosciences. 2015.4(4) ,2072-2076.
[15]. FengShuang, Ma Shurong. Advances in germination of desert woody plants under salinity stress. Food and Agricultural organization of the United States. 2013.

Patil N.M. "Effect of different Pre sowing treatments on seed germination of endangered medicinal plant Caesalpiniabonducella (L) Roxb. "IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 13.3 (2018): 54-59