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Research article

MULTIPLE SHOOTS AND ROOTS OF GERMINATING *SARACA ASOCA* SEEDS

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ABSTRACT: This study, on the occurrence of seed storage temperature mediated multiple seedlings, explains the multiple shoot and root formation in *S. asoca* during germination apart from that of the previously attributed polyembryonic lineage.

Key words: *Saraca asoca*, multiple seedlings, storage.

INTRODUCTION

Saraca asoca (Roxb.) W.J. de Wilde of the subfamily Caesalpiniaceae is a medium sized evergreen tree distributed in India, Sri Lanka, Bangladesh, and Myanmar [1]. IUCN has red listed this species under the threat category "globally vulnerable" [2]. There have been reports on poor seed set in natural populations of the Western ghats and difficulties in large scale propagation of 'asokam'through seeds [3]. The sweet kernel of seeds are predated by insects, wild boars [1] monkeys and languors [4]. Desiccation sensitive seeds of *S. asoca* germinate quickly during rainy season at the moist forest floor of tropical rain forest zones. *S. asoca* seeds on germination also produce relatively low proportion of multiple seedlings, reason of which was reported as polyembryony [5]. Generally the seed germination related abnormalities were categorized in to double embryo, polyembryony, twin and triple seedlings, albino and chlorophyll mutant seedlings *etc*. [6]. Troup [7] gave a comprehensive account of seeds and seed germination in respect of Indian forest species but did not mentioned about normal or abnormal seedlings. Observations of Wieringa and Leendertz [8] and Sebelin [9] on the morphological development of seedlings during the course of germination evolved a key of normal and abnormal seedlings [10]. Gunanga and Vasudeva [6] reported existence of such abnormal seedlings in several tropical tree species though their growth performance at juvenile stages were not reviewed much. In this background, the present study evaluated the formation and pursuance of multiple shoots or roots during germination of *S. asoca* seeds.

MATERIALS AND METHODS

Seed collection

Mature dry dehiscent pods were collected during Jan-May of three consecutive years 2010-2013 from JNTBGRI campus at an altitude of 150 m, amongst the foothills of southern Western Ghats (Lat-$8^0$45' and $8^0$47'N; Long-$77^0$1' and $77^0$4'E). Mature fruits depicted by their well-developed abscission zone and characteristic dark brown color were collected from all ten trees were pooled into a single lot.

Effect of different storage temperatures on abnormal germinants production

Saraca asoca seeds were hermetically stored in polycarbonate bottles up to one week at different ambient temperatures like 30, 20 and 10^{o}C for analyzing the effect of multiple seedlings emergence. Seeds without pretreatment were considered as control. Both control and stored seeds with ten replicates of ten seeds each were germinated in rolled acid free germination papers kept in a seed germinator without light (30±2^{o}C, 80% RH). The seeds were scored as germinated when the radicle came out to a length of 5 mm [11]. Germination percentage of seeds were the collective results of normal and abnormal germinants.

Seedling performance

Multiple seedlings with twin and triple shoots were selected for comparative analysis of seedling performance in a greenhouse with 50 % shade along with normal seedling up to the period of six months. Various seedlings traits *viz.* number of leaves, number of leaflets/leaf, seedling height (from soil surface to the highest leaf apex), stem height (from soil to the tip of the stem), shoot height (from soil to the first leaf petiole), stem diameter (at soil level), maximum root length and maximum root diameter were recorded. Dry mass was measured separately for the root and shoot of seedlings which were dried to constant mass in paper bags in an oven at 70^0C.

Statistical analysis

All experimental data were statistically analyzed by one way ANOVA and the values are expressed as mean ± standard deviation. Significance of differences between means were tested by LSD ($P < 0.05$& 0.01; n = 10).

RESULTS AND DISCUSSION

Recalcitrant seeds of *S. asoca* are non endosperms with two cotyledons and an embryonic axis covered with thin seed coat. Two cotyledons serve as sole food storage organ, which covers around 95.23 % of whole seed. Normally *S. asoca* seeds produce single plumule (shoot) and radicle (root) from embryonic axis during hypogeal germination. At laboratory condition, our observations revealed that 2 % of early sprouts develop multiple seedlings of two to three shoots per seed (Figure 1B & 1C). Similar observation was reported with riparian species of *Lophopetalum wightianum*, an invariable component of the endangered *Myristica* swamp ecosystem of Western Ghats [12]. Wanage *et al* [5] observed up to five shoots in *S. asoca* with reason been attributed to polyembryony.

Multiple seedlings of *S. asoca* usually lacks equal number of shoots and roots, which juxtaposed with results of conjointed twin and triplet polyembryonic seedlings of *Salacia chinensis* [13] , *Juniperus polycarpos* [14] *and Amla* [15]. The twin seedlings derived from two embryos per seed reported in *Juniperus polycarpos* [14] and *Rauvolfia serpentine* [16] were found to be independent, having separate taproots and cotyledons. The guggul plant (*Commiphora wightii*) clearly shows multiple embryos in a single seed [17] by the effect of polyembryony [18, 19] which differs to the *S. asoca* seeds, because seeds of *S. asoca* are monoembryonic with two cotyledons and an embryonic axis. In *S. asoca*, inseparable form of multiple seedlings were observed that may be directly developed from two apical primordia of single embryo having the potential to form meristem. Weather wax [20] reported the occurrence of multiple shoot and root during germination in maize grains and states that the disturbance of embryonal axis polarity during differentiation was the reason for anomalous embryo, which derived from a single fecundated egg. Internal disturbance of seed tissues through either physiological and biochemical or at genetic levels are highlighted as reason for abnormal germinants in *Azadirachta indica* [21]. Generally seed coat act as primary defense shield against adverse environmental conditions to protect the embryo from microbial infection and deterioration [22]. After water imbibition, cotyledons of *S. asoca* were slightly push aside by the rupture of thin seed coat thus meristammatic zones of partially exposed embryonic axis was seemingly prone to either biotic or abiotic damages. Injury imparted on single hypocotyl axis of *Bruguiera gymnorrhiza*, a mangrove species through decapitation method effectively induced multiple shoots at *in vivo* condition [23]. In another case mutation of the FACKEL (FK) gene affected the body organization of the *Arabidopsis* seedling and resulted in the formation of seedlings with multiple apices or seedlings with multiple roots [24]. Histological observations on rice mutants (OsCem) that produce multiple plumules and/or radicles indicated their origin from ventral and dorsal sides of the same embryo [25]. The auxin polar transport inhibitors N-1-naphthylphthalamic acid and quercetin induced a number of specific abnormal phenotypes in wheat embryo culture, on which Fischer *et al* [26] suggested that the polar transport of auxin has a determining influence on the differentiation of extra root or shoot meristems.

Table.1: Relation between storage temperature and induction of multiple seedlings

Storage (one week in polycarbonate bottle)	Percentage of multiple seedlings	Total germination percentage (includes both normal and abnormal germinants)
Control	2.0±0.52	100
30^0C	6.0±3.06*	100
20^0C	9.0± 3.14**	100
10^0C	10.0±3.33*	100

Results are represented as means (±SE) in the column with (**) (*) (ns-not significant) according to LSD at the 5 % and 1 % levels ($P< 0.05$&0.01; n=10)

In this study, it was found that different storage temperatures significantly induced multiple seedlings production in *S. asoca* (Table 1), akin to test with soyabean [27] and eucalyptus seeds [28]. *S. asoca* seeds kept at 10^{o}C produced more number of radicles (Figure 1D) but other temperature treatments prefer shoot multiplicity. Apart from air pressure, Gardiner [28] reported the impact of low temperature on seed germination abnormalities during storage. Low temperature related seed damage [29] and reduction of apical dominance [30, 31, 32, 33] are the probable reasons of multiple shoots production in peach cultivars [34]. Embryo cryopreservation [35] also may stimulate formation of abnormal seedlings.

Table.2: Field performance of seedlings

Seedling characters	Normal seedling		Seedling with twin shoot		Seedling with triple shoot	
	Three months	Six months	Three months	Six months	Three months	Six months
Number of leaves	2.3±0.15	5.1±0.35	2.6±0.31 ^{ns}	4.4±0.37 ^{ns}	2.3±0.15 ^{ns}	4.62±0.22 ^{ns}
No.leaflets/leaf	2.4±0.27	3.6±0.27	2.4±0.27 ^{ns}	2.8±0.33 ^{ns}	2.4±0.27 ^{ns}	3.2±0.33 ^{ns}
Seedling height	15.06±0.35	21.85±1.66	13. 61±0.50 ^{ns}	22.34±1.30 ^{ns}	16.03±1.12 ^{ns}	16.03±1.12 ^{ns}
Stem height	11.67±0.70	18.30±0.86	12.22±0.47^{ns}	21.57±1.16*	14.86±0.29**	15.35±1.03 ^{ns}
Shoot height	12.89±0.58	19.44±0.99	12.89±0.46 ^{ns}	22.34±1.15 ^{ns}	13.37±0.58 ^{ns}	15.4±1.05 ^{ns}
Stem diameter	0.27±0.02	0.54±0.01	0.21±0.01 ^{ns}	0.38±0.01 ^{ns}	0.15±0.01 ^{ns}	0.32±0.02 ^{ns}
Root max. length	11.00±0.82	18.3±1.50	15.25±0.54**	24.80±1.06**	16.78±0.59**	24.50±1.46**
Root max. diameter	0.35±0.02	0.73±0.02	0.36±0.01 ^{ns}	0.66±0.01 ^{ns}	0.27±0.01 ^{ns}	0.46±0.02 ^{ns}
Root dry mass	0.97±0.09	1.44±0.15	0.81±0.07^{ns}	1.31±0.07 ^{ns}	1.09±0. 09^{ns}	1.76±0.07*
Shoot dry mass	0.80±0.08	2.22±0.10	0.78±0.10 ^{ns}	3.64±0.16**	0.54±0.05 ^{ns}	3.24±0.14 **

Results are represented as means (±SE) in the column with (**) (*) (ns-not significant) according to LSD at the 5 % and 1 % levels (P< 0.05&0.01; n=10)

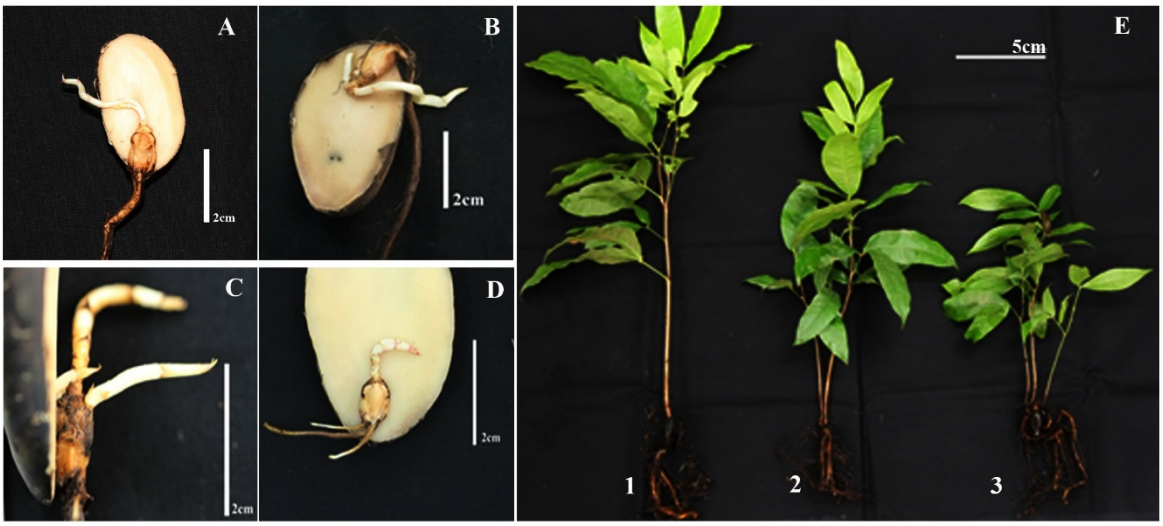

Figure 1: A- normal & (B-D) abnormal seed germinants with two plumule (B); three plumule (C), three radicle (D); E- six month old seedling of (1) control, (2) two plumule and (3) three plumule

Seedling traits include leaf number, stem/seedling/shoot heights, stem/root diameter and root dry mass were differed among seedling classes, but not much significant (Table 2). The normal seedlings showed an average seedling height of 21.85 cm at the age of six months, twin shoot seedling height was almost same while triple shoot seedlings showed slower growth as compared to normal and twin shoot seedlings. The short stature of triple shoot seedlings may be due to the competition between seedlings for the maternal resources [36]. Root maximum length and shoot dry mass are traits which shows significant difference (P > 0.05 & 0.01) from normal seedlings. Increase in root length may be a response to competition, leading to growth to soil depths where root density would be smaller [37].

The average root length of both class of seedlings were higher with that of control for better nutrient availability. Altogether multiple seedlings of *S. asoca* could be grouped under deformed or unbalanced type, subclass of abnormal category because of those seedlings did not showed the potential to develop into a normal plants when grown in soil under favorable conditions of moisture, temperature and light [38] (Figure 1E). On contrary, polyembryonic seedlings of as reported in the case of *Rauvolfia serpentina* showed more vigour than normal seedlings at field conditions [16]. It is inferred that *S. asoca* multiple seedlings are not polyembryonic origin since their vigour is lesser. Hence there is sufficient scope for research in this direction that has to be studied separately in order to establish the categories of abnormalities inherent within the seeds of a species.

CONCLUSION

At *in vivo* condition, polyembryos were artificially induced in the flower buds of Capsicum species on treatment with different growth regulators (2,4-D, BNOA & BAP) [39].This highlights that the polyembryos induction was achieved only through pre or during embryogenesis phase of seed development, which is unlike that of the previous assumption of linking with polyembryony in *S. asoca*. Observations regarding poor field performance and inseparable formation of multiple seedlings also supports features of abnormal seedlings rather than of polyembryonic ones. Effect of hypocotyl decapitation as reported in mangrove species [23] unwrap possibility of mechanical damage which may act as stimulant for multiple shoot induction either control or storage environments in *S. asoca*. Alteration on embryo axis pattern and auxin polar transport are considered as other reasons for multiple plumule or radicle in rice mutant (OsCem) [25], which also substantiate the probable occurrence of multiple seedlings of *S. asoca* though this need further studies of genetic analysis and molecular mapping for confirmation. The local auxin gradient produced by polar transport represents a common module that operates in the formation of all plant organs, regardless of their mature morphology or developmental origin [40]. In line to this report, term polyembryony is not suitable for the seedlings of *S. asoca*, instead the occurrence of multiple shoot or roots be explained by internal disturbances of seed tissues either due to physiological, biochemical or genetic factors.

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