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Estimation of Primary Metabolites and Phenols in the Seeds of Five Species of *Calamus* and their Role on Germination

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ABSTRACT

Background and Aim: *The mass scale harvest of Calamus before flowering and fruiting resulted in the depletion of precious germplasms and shortage of required seed for further propagation. Seeds of Calamus species are recalcitrant and cannot withstand desiccation, a challenge for the storage. Correlation between biochemical traits and germination contribute to the better understanding of seed dormancy and to assess the germination capacity of the given taxon.*

Materials and Methods: *Carbohydrates, proteins, amino acids and phenol contents were estimated in the seeds of Calamus heugelianus, C. prasinus, C. nagabettai, C. thwaitesii and C. vattayila for the first time following standard biochemical methods.*

Results: *The contents have shown variations among the taxa studied. Carbohydrate content of 8.25 ± 0.04% is significantly high in the seeds of C. prasinus than other species studied. While higher content of proteins (8.92 ± 0.02%) and amino acids (9.36 ± 0.02%) were found in the seeds of C. heugelianus. The phenolics content was found to be more in the seeds of C. nagabettai than other taxa studied. The levels of all the studied bio constituents are minimum in C. vattayila. Effect of the storage macromolecules on germination behaviour of the seeds is discussed.*

Key words: *Calamus, Germination, Phenols, Primary Metabolites, Seeds.*

INTRODUCTION

Calamus, one of the important tropical non timber products whose stems are being used as canes in furniture industry is belongs to the family Arecaceae. Habitat destruction, over exploitation and unscientific harvesting methods for the past few decades have resulted in a drastic depletion of the *Calamus* resources. Though *Calamus* species produce abundant fruits during the season, there is a difficulty in supplying good quality seeds to the cultivators because of untimely harvesting of the canes.

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Calamus species are usually propagated by seeds. Seeds act as sinks for various metabolites synthesised during their development which includes both embryogenesis and endosperm differentiation. Important storage metabolites are carbohydrates, proteins, amino acids and phenols which are directly linked to germination[1, 2]. Wide variation in germination pattern in various seed lots is attributed to the presence or absence of particular metabolite content in the seeds[3, 4]. In general, seeds of *Calamus* take longer time to germinate. Percentage germination in *in situ* is affected by soft sarco testa which leads to the decay of the seeds and prolonged dormancy leads to predation, drying and diseases[5]. Though studies on germination and seedling vigour were initiated since 1970s, information on the biochemical constituents in the seeds is lacking[5-13] .Since studies on biochemical traits in seeds provide an insight in to the understanding of the phenomena of seed dormancy and germination characteristics, the present work is an attempt to estimate bio constituents in the seeds of following five species of *Calamus* which are economically important and to correlate the data with the germination studies done by us[13] and others.

Calamus heugelianus, an endangered taxon, is a clustering cane with the stem growing up to 30 m or more. The seeds of this taxon are reported to be highly recalcitrant and not responded to any pre-treatment to enhance the percentage germination[14]. Dormancy period is considerably long and taken more than three months to germinate and percentage of germination was also below 50% on various substrates[14].

Calamus nagabettai, an endemic species to Karnataka is a clustering cane with stem growing up to 25m or more. The seeds of this taxon are also recalcitrant and not responded to any pre-treatment tried to enhance the percentage of germination[13].

Clamus prasinus is a solitary high climbing cane which is endemic to Karnataka. The seeds are highly recalcitrant and MGT varied from 120 to 150 days. However, percentage of response on various substrates is significantly more than the previous two species[13].

Calamus thwaitesii is a robust, clustering cane with the stem growing up to 20m or more. GA_3 treated seeds have shown better response in terms of germination. MGT also is less than 30 days in GA_3 treated seeds and significantly low on various substrates[13,15].

Calmus vattayila is a high climbing cane with solitary stem. GA_3 treated seeds have shown better percent germination on paper bridge method. Percent of germination and vigour index were also significantly better in GA_3 treatment and on various substrates[13].

MATERIAL AND METHODS

Mature seeds of the selected taxa were collected from tropical forest of Subramanya range in Western ghats, India. Mature seeds of each species were collected from randomly selected twelve plants from their habitat- Western ghats. Thus collected seeds are pooled and sun dried. 100 healthy seeds of each taxon were selected randomly in five replicates and used for each of the different biochemical estimation. The seeds were powdered with pestle and mortar. Samples were prepared with aqueous, ethanol, methanol, and petroleum ether and chloroform solvents as required for further studies. The contents of bio constituents are expressed in percentage (%) and tabulated.

1. Estimation of carbohydrates

Carbohydrate contents were determined by anthrone method of Yemm and Willis[16]. 200 mg of the seed powder of five samples was homogenized in 10 ml of 80% methanol. The homogenate was centrifuged at 10, 000 rpm for 10 min. The supernatant was retained and final volume was made up to 10 ml. To 1 ml of this sample, 4 ml of anthrone was added and heated in a water bath for 8-10 min. The tubes were cooled and the absorbance of the sample was read at 630 nm. Standard graph was prepared with different concentrates of glucose.

2. Estimation of proteins

Protein content was estimated by following Lowry's method[17] using Bovine serum as standard. 200mg of seed powder of selected 5 taxa were homogenized in 10ml of 1.5M NaCl under the cold condition. The homogenate was centrifuged at 10, 000 rpm for 10 min and 10 ml of 10% TCA was added to supernatant. The precipitate was dissolved in 1N NaOH and made up to 20 ml. The absorbance was read at 660nm using Bovine as standard.

3. Estimation of amino acids

The content of amino acids was estimated following Ninhydrin method[18]. 200 mg of the seed powder were homogenized in 10 ml of 80% ethanol. The homogenate was centrifuged at 10, 000 rpm for 10 min. The supernatant was collected and used for the quantification of total free amino acids with glycine as standard.

4. Estimation of phenols

200 mg of seed powder was homogenized in 10 ml of 80% ethanol and homogenate was centrifuged at 10,000 rpm for 10 min. The supernatant was evaporated to dryness. The residue was dissolved in 5 ml of distilled water and phenols were estimated following Singleton and Rossi[19] using catechol as standard.

Data analysis

The results thus obtained were expressed in percentage (%) and data was tabulated (Table 1). The data obtained from the experiments were subjected to one-way analysis of variance (ANOVA). Significant F ratios between the group means were separated by Duncan's multiple Range Test (DMRT) using SPSS version 1.5. Probability values <0.05 were considered as significant[20].

RESULTS AND DISCUSSION

The Information on the nature and extent of seed source variation with regard to important primary metabolites like carbohydrates, proteins and amino acids that are directly linked to germination[1,2] is essential for genetic improvement of a species[21], especially for early selection. There are reports that have attributed the wide variation in germination to differences of particular metabolite contents of various seed lots[3]. However, Rawat and Uniyal[22] have found no correlation among the studied biochemical parameters and percentage germination in conifers.

Carbohydrates, proteins, amino acids, lipids and phenols are the major macromolecules in the seed which control the germination pattern. The seeds of *Sterculia urens* were rich in proteins, carbohydrates, lipids and their levels decrease as the germination progress indicating their key role in the growth of embryonic axis[23]. Carbohydrate contents in the samples studied in the present investigation have shown wide variations ranging from 2.95 ± 0.05 to 8.25 ± 0.04. Maximum amount of 8.25 ± 0.04 was recorded in *C. prasinus* followed by *C. nagabettai* with 4.92 ± 0.01, *C. heugelianus* with 4.73 ± 0.02 and 4.21 ± 0.02 in *C. thwaitesii*. Least amount of 2.95 ± 0.05 was recorded in *C. vattayila* (Fig.1). It has been demonstrated that soluble carbohydrates play a key role in desiccation tolerance and seed storage[24]. Increase in levels particularly in the contents of sucrose of the oligosaccharides has been correlated with desiccation tolerance and longevity[24,25]. Results of the present study indicated significant differences among the various taxa studied in qualitative biochemical parameters. Carbohydrate content in the seeds of *C.prasinus* was found to be more than other taxa studied in the present investigation. Tejavathi et al.,[13] have reported 93% of germination in this taxon on vermiculate and 86 in soil. However time taken for germination exceeds one year. Germination capabilities of seeds depend on desiccation stage where concentration of certain organic compounds including increase of sucrose and amino acids act as physiological factors and / or chemical promotions via various internal germination mechanisms[26]. Least amount of carbohydrates was recorded in the seeds of *C. vattayila* where 90 percent of germination was recorded by Tejavathi et al.,[13] under normal conditions and MGT was only 4 days.

Protein contents in the seeds of selected species in the present study were varied from

8.92 ± 0.02 to 4.33 ± 0.04. Maximum content of 8.92 ± 0.02 was recorded in *C. heugelianus*, next best was found in *C. nagabettai* (8.60 ± 0.05) followed by *C. thwaitesii* (8.25 ± 0.01) and *C. prasinus* (4.68 ± 0.04). However, least amount of 4.33 ± 0.04 was found in *C. vattayila* (Fig 1). Vast majority of individual proteins present in mature seeds have either metabolic or structural role. Seeds contain one or more groups of proteins that are present in high amount and that serve to provide or store of amino acids for the use during germination and seedling growth. These storage proteins are of particular importance because they determine not only the total protein content of the seed but its quality. Upon germination these proteins are mobilized to provide nutrients for seedling growth[3]. Seeds of *C. heugelianus* contain maximum content of amino acids (9.36 ± 0.02). Least amount of amino acids was noted in *C. vattayila* (6.40 ± 0.02). While *C. nagabettai* and *C. thwaitesii* contain 9.21 ± 0.02 and 8.76 ± 0.02 of amino acids respectively (Fig.1). However, *C prasinus* seeds contain 6.52 ± 0.02 of amino acids. Though the seeds of *C. heugelianus* contain more levels of protein and amino acids than other taxa studied, the percentage germination and MGT recorded by Tejavathi et al.[13] (2013) in this taxon are comparatively less. Kanmegne et al.,[27] have reported negative correlation between the protein content of seeds and germination. The seeds of accessions of *Garcinia kola* with low protein content germinated better than the seeds with high protein content. This result which contradicts that of Bhatt et al.,[28] on Irish potato seeds and also Lee et al.,[29] on *Prunus companulata* where protein content increases when the dormancy period is released. It is widely known that a key factor in seed germination is the hydrolysis of storage proteins[30]. However, dormancy related proteins have been identified in *Arabidopsis*[31,32]. Protein like RGL2DELLA, which is associated with dormancy, disappears after 5 hr dormancy-breaking treatment in *Arabidopsis* suggested that a quick turnover of this repressor induce germination. The recorded data in the present studies shows that high protein content in the seeds of *C. heugelianus* may be representing dormancy related proteins that can be correlated to less percent of germination as reported by Tejavathi et al. Kanmegne et al.,[27] had attributed the high content of proteins in the seeds of *Garcinia cola* to dormancy related proteins as the seeds have shown less percent of germination. Least amount of protiens (4.33 ± 0.04) has been reported in *C. vattayila* which can be correlated to significantly less MGT, better percent germination and seedling vigour compared to other taxa studied[13].

Like primary metabolites, total phenol contents also shows variation among the samples. 8.55 ± 0.01 was the highest record for *C. nagabettai* among the samples studied, followed by *C. heugilianus* (8.25 ± 0.01) and *C. thwaitesii* with 8.14 ± 0.01. Next best record was noted in *C. prasinus* with 7.65 ± 0.01 (Fig. 1). However least record of 7.51 ± 0.01 was seen in *C. vattayila*. Phenolic compounds are wide spread in their distribution in seeds, fruit and other plant tissues. Various studies have shown that phenolics are inhibitory in germinating seeds and also for the growth[33]. Seeds of *C. nagabettai* contain more phenolics than the other taxa studied. Tejavathi et al.[13] have however reported less percentage of germination and long period of dormancy in *C.nagabettai* compared to other taxa studied. Studies on elucidation of the biochemical changes during desiccation of *Calamus rotang* and *C. thwaitesii* revealed that hydrolysis of carbohydrates, degradation of proteins and accumulation of phenolic substances in the seeds during desiccation resulted in the death of the seeds[34]. Least amount of phenolics was recorded in the seeds of *C. vattayila* of the present investigation that has reflected in high percentage of germination in this taxon compared to others in all the treatments[13]. Germination was inhibited when seeds were treated with phenolics in soybean[35] and some crop plants[36, 37]. Phenolic compounds present in the soil have inhibited the germination of the seeds of *Quercus* [38]. Studies on the

Phenolic compounds present in the seeds of *Palicourea rigida* indicate that phenolic compounds are inhibitors of germination thereby enhancing the dormancy period by inhibiting the activities of peroxidises[39].

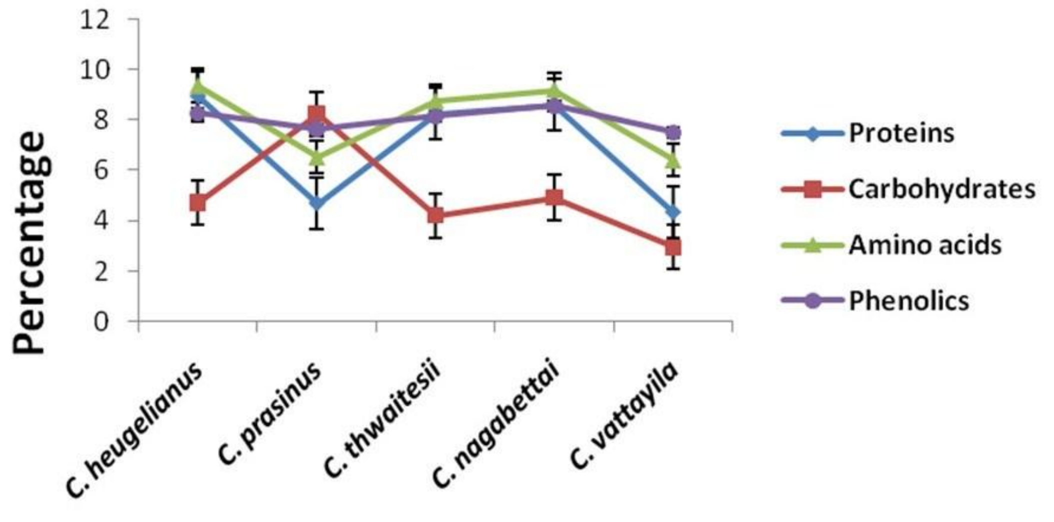

Fig. 1. Estimation of Metabolites

CONCLUSION

It can be concluded from the aforesaid data that there is a correlation between the accumulated storage macromolecules and the germination characteristics in a given taxa. Knowledge about the bio constituents in the recalcitrant seeds is a prerequisite prior to employ any strategy for storage of seeds and thereby conservation of the germplasms of the threatened taxa likes *Calamus.*

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