THE EFFECT OF SPECIFIC GRAVITY SEPARATION ON GERMINATION AND BIOCHEMICAL POTENTIAL OF *CASUARINA EQUISETIFOLIA* SEEDS

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UMARANI, R. & VANANGAMUDI, K. 2002. The effect of specific gravity separation on germination and biochemical potential of *Casuarina equisetifolia* seeds. Empty seeds and dormancy are the two factors that prohibit germination of live seeds. *Casuarina equisetifolia* shows only 40 to 45% germination even when they are freshly collected. To see if seed filling can be a cause for this dismal record of germination, an attempt was made to separate the seeds based on the weight of the individual seed using a specific gravity separator. The results revealed that seed germination recorded by A grade seed was significantly higher than B and C grades as well as ungraded bulk seeds. D and E grades did not germinate at all. The estimation of storage reserves revealed that variation existed among the grades separated by the specific gravity separator. The A grade seeds recorded higher protein, oil and carbohydrate contents, which substantiated the higher germination and vigour recorded by this grade of seeds.

Key words: Empty seeds - dormancy - grade - gravity separator

UMARANI, R. & VANANGAMUDI, K. 2002. Kesan pemisahan graviti spesifik terhadap percambahan dan potensi biokimia biji benih *Casuarina equisetifolia*. Biji benih kosong dan kedormanan merupakan dua faktor yang menghalang percambahan biji benih hidup. *Casuarina equisetifolia* menunjukkan hanya 40 hingga 45% percambahan walaupun biji benih tersebut baru dikutip. Untuk melihat sama ada pengisian biji merupakan sebab kejayaan percambahan yang rendah ini, cubaan dibuat untuk mengasingkan biji benih berdasarkan berat setiap biji benih menggunakan pemisah graviti spesifik. Keputusan menunjukkan percambahan yang dicatatkan oleh biji benih gred A lebih tinggi dengan bererti berbanding biji benih gred B dan C serta biji benih pukal yang tidak digredkan. Biji benih gred D dan E tidak bercambah langsung. Gred biji benih yang diasingkan oleh pemisah graviti spesifik menunjukkan jumlah nutrien yang berbeza-beza. Biji benih gred A mencatatkan kandungan protein, minyak dan karbohidrat yang tinggi, mengesahkan bahawa percambahan dan kecergasan yang tinggi dicatatkan oleh biji benih dalam gred ini.

Introduction

Casuarina equisetifolia seeds record only 40 to 50% germination even when freshly collected. Live seeds fail to germinate either because of dormancy or due to emptiness. Seeds of *C. equisetifolia* are small-sized samaras, feathery and light weighted. These seeds do not sink in water no matter how long they are soaked. Hence, separation of high density seeds through water floatation technique is not possible. Specific gravity separator stratifies the seed grades based on the weight of individual seeds, so as to separate the filled seeds from the empty seeds. Specific gravity separation has been utilised to select *Eucalyptus* seeds with higher weight and to increase seed germination percentage (Dharmalingam *et al.* 1973, Khan 1976).

Protein, carbohydrate and lipids constitute the bulk of the storage reserves in seeds (Bewley & Black 1983). Mobilisation of these reserves occurs during germination and the early stages of seedling growth. The breakdown products are used by developing seedlings as a nutritive source (Anderson & Abdul-Baki 1971).

In this present study, seeds of *Casuarina equisetifolia* were separated into five grades using a specific gravity separator. The specific gravity separator worked on the principle of floatation in air to stratify and separate seeds according to the specific gravity of seeds. The grades thus separated were analysed for differences in seed viability and vigour. The protein, carbohydrate, reducing sugars and oil content were tested to understand the basis of differences in seed germination.

Materials and methods

Seeds were extracted from fresh cones of *C. equisetifolia* collected from Coimbatore District (11.00° N, 77.00° E) of Tamil Nadu, India. Seeds were graded in a specific gravity separator (WESTRUP, LA-K No. 89036) with a vertical height, horizontal height and air blow rate adjustments of 2, 0 and 3 respectively at 390 to 410 rpm (Umarani 1999). The seeds were upgraded accordingly into five grade classes, namely, first, second, third, fourth and fifth, designated as A, B, C, D and E respectively. The following estimations were made from the different specific gravity grades of seeds:

Germination test

The seeds were surface sterilised with 0.01% (w/v) mercuric chloride for 3 minutes and washed thoroughly with distilled water. A total of 100 seeds of each grade class were set in roll towel method (Anonymous 1995). The germination percentage, recorded as seeds that produced normal seedlings, was determined after 14 days of sowing. After germination count, 10 random seedlings were measured for their root and shoot lengths and vigour index (germination (%) × seedling length (cm)) (Abdul-Baki & Anderson 1973) was derived. Ten random seedlings were kept in an oven maintained at 80 °C for 24 hours. Later the samples were cooled in a desiccator, weighed and expressed in mg per10 seedlings. The experiment was completely randomised and replicated three times.

Protein

A sample of 100 mg of seed material, after 48 hours of imbibition, was homogenised in 1.8 ml of 0.02 M sodium phosphate buffer (pH 6.0). The protein content was measured following the protein-dye binding method (Bradford 1976).

Carbohydrate

Seed material weighing 100 mg was hydrolysed for three hours with 5 ml of 2.5 N HCl and neutralised with sodium carbonate. The supernatant obtained after centrifuging was subjected to Nelson-Somogyi (Nelson 1944) method and the carbohydrate content was estimated.

Reducing sugars

Ethanol extract was prepared by re-extracting twice from 100 mg of seeds. The reducing sugar content was estimated using the method by Nelson (1944).

Oil

Oil was extracted from the seeds with petroleum ether for 3 hours in a soxhlet by gentle heating.

All results obtained from the tests were subjected to analysis of variance and tested for significant differences (Panse & Sukhatme 1978).

Results and discussion

The data obtained showed that the five grades of seeds differed significantly for seed germination percentage, seedling vigour and biochemical status (Table 1). The A grade seeds recorded the highest filling percentage (95%) with a 100 seed weight of 131 mg. The B,C,D and E grades recorded 73, 38, 17 and 10% of seed filling corresponding to 85,79,72 and 70 mg of seed weight respectively.

Table 1 Effect of specific gravity of seeds on seed filling and hundred seed weight (mg) of *Casuarina equisetifolia*

Specific gravity class	Seed filling (%)	100 seed weight (mg)
A	95	131
B	73	85
C	38	79
D	17	72
E	10	70
Bulk	82	129
Mean	52	94
CD (p = 0.05)	3.08	2.60

The percentage of seed germination recorded by A grade seeds was 30.4. This value was 50.6, 88.4 and 14.4% higher than the B and C grades and ungraded bulk seeds respectively (Figure 1). D and E grades did not germinate at all. The seedling growth and vigour also followed the same trend as seed germination.

Among the biochemical parameters, the protein content was less in B, C, D and E grades and bulk seeds with respect to A grade seeds. The percentage of decrease for B, C, D, E grades and bulk seeds compared with A grade were 27.9, 52.4, 71.9, 71.9 and 49.9% respectively. Similarly, the decrease in oil content in B, C, D, E grades and bulk seeds compared with A grade seeds was 14.6, 30.3, 69.9, 95.7 and 16.9% respectively (Table 2).

Unlike the protein and oil content, the carbohydrate and reducing sugar contents progressively increased from A to E grades. The increases in B, C, D, E and ungraded bulk over A grade were 13.1, 17.1, 27.6, 36.8 and 19.7 respectively. Reducing sugar content also showed a similar trend; the percentages of increase for reducing sugar compared with grade A seeds were 0.05, 0.20, 0.71, 0.76, 1.10 and 0.28% respectively for A, B, C, D, E grades and the ungraded bulk.

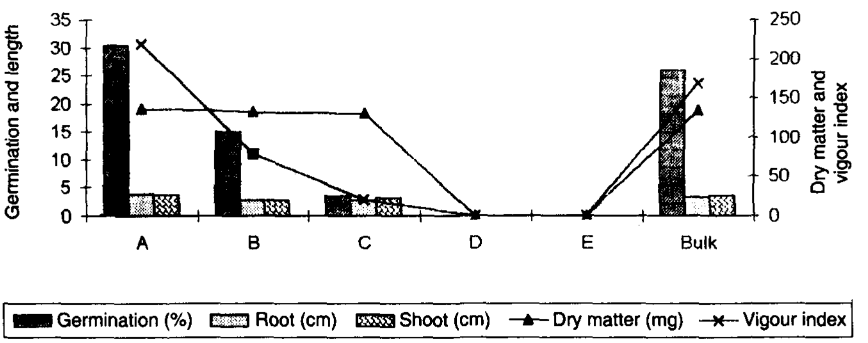

Figure 1 Effect of specific gravity of seeds on germination (%) and seedling growth of *Casuarina equisetifolia*

Table 2 Effect of specific gravity of seeds on protein, carbohydrate, reducing sugar and oil content (%) of *Casuarina equisetifolia*

Specific gravity class	Protein (%)	Carbohydrate (%)	Reducing sugar (%)	Oil (%)
A	0.427	7.6	0.05	20.96
B	0.308	8.6	0.20	17.90
C	0.203	8.9	0.71	14.60
D	0.120	9.7	0.76	6.30
E	0.120	10.4	1.10	0.90
Bulk	0.214	9.1	0.28	17.40
Mean	0.232	9.05	0.52	13.01
CD (p = 0.05)	0.008	0.029	0.09	0.390

The data obtained in the present study showed that protein and oil content decreased as quality of seeds decreased. However, an inverse trend was visible for carbohydrate and reducing sugar contents. The observation of the filling percentages showed that as the seed quality decreased the filled seed percentage also decreased. That is, A grade seed, which recorded 95% filling, contained more of true seed whereas, with the decrease in seed quality from B, C, D to E, the filling percentage also decreased. This showed that as the seed quality decreased the seed sample comprised more of husk (pericarp) and not the true seed. The sugar content, either total sugars (carbohydrate) or reducing sugars, increased with the increase in the percentage of husk. Hence, it was possible to infer that carbohydrate was contributed mainly by the husk (pericarp) and not the seed itself, whereas the rest of the estimates, i.e. protein and oil, were more a part of the true seed than the husk.

The superiority of the A grade seeds in seed filling and subsequently in the hundred seed weight as well as protein and oil contents, explained the increased germination and seedling vigour in these seeds. Several authors have recorded their experience on the positive influence of upgrading the seed quality on the subsequent seed germination and seedling vigour. Seed upgrading usually entails removal of empty, immature, broken or insect damaged seeds. After extraction and cleaning seed lots should be further conditioned to upgrade the quality of the lots. Significant upgrading of *Platanus occidentalis* by removal of empty seeds has been reported (Bonner & Switzer 1971). Upgrading of *Eucalyptus* seeds on specific gravity basis by using water floatation method or gravity separator significantly increased the seed weight and germination (Dharmalingam *et al.* 1973, Khan 1976). Density grading of depulped neem drupes using water has produced better seedling production (Ponnuswamy 1993).

Even though carbohydrate forms the largest component in acorns, lipid still may be a critical food reserve (Clatterbuck & Boner 1985). Protein is the main form of nitrogen storage in the seeds. Utilisation of protein and lipid after germination is rapid in seedling. Digestion of the protein begins immediately upon hydration of the seed and this is followed quickly by hydrolysis of lipid (van Staden & Brown 1997).

This study proved the importance of specific gravity separation to obtain seeds with high protein and oil content. It is hence concluded that seeds of *C. equisetifolia* should be separated based on their specific gravity. By adopting this technique the quality of the seed lot, with respect to seed germinability and seedling vigour, can be improved.

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