https://doi.org/10.29321/MAJ.10.A00100

The effect of specific gravity separation on germination and enzyme activity of *Casuarina equisetifolia* seeds

UMARANI AND K. VANANGAMUDI
est College and Research Institute, Mettupalayam - 641 302, Tamil Nadu

Abstract: An attempt was made to separate the seeds based on the weight of the individual seed by using the specific gravity separator. Seed germinaton recorded by A grade seeds was significantly higher than B, C grades and ungraded bulk seeds. D and E grades did not germinate at all. The superiority of A grade seeds was also observed in enzyme activity viz. amylase, catalase, peroxidase and superoxide dismutase explained the increased seed and seedling vigour in the A grade seeds.

Key words : Casuarina equisetifolia, Seed quality, Specific gravity grading, Enzyme activity, Germination.

Introduction

Casuarina equisetifolia is an important plantation tree which meets the requirements of rural folk for fuel, fodder, poles for farm constructions and shelter belts in areas of high winds. It also serves a source of pulp for manufacture of paper and rayon. Further, the tree has high biological significance, owing to 20-25 tonnes per ha of biomass of leaf litter, shed by the tree. The seeds are the main mode of propagation, however the germination of fresh seeds is very meagre *i.e.* 40-50%. The differences in seed density due to variations in seed filling can be exploited to separate the good quality seeds. Amylase is involved in supply of assimilable sugars for seedling growth. Superoxide dismutase is a free radical scavenger (Frank, 1985). Catalase and peroxidase act as protector against accumulation of peroxide (Woodstock, 1967). These three enzymes are imperative for the viability of seeds in storage. The enzyme activity in a seed is a dependable biochemical index of seed viability and vigour.

Against these back drops, the five different seed grades obtained from the specific gravity separator were observed for their germination performance. The seeds were also analyzed for the activities of enzymes viz. amylase, catalase, peroxidase and superoxide dismutase to correlate the seed grade with it's physiological and biochemical potential.

Materials and Methods

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

1. Seed filling

The true seeds were picked out from the samaras using a dissection needle, the number of seeds filled was recorded and expressed as percentage of seed filling.

2. Hundred seed weight

Determination of hundred seed weight was computed as per ISTA (1985). For this 8x100 seeds were counted at random from the seed sample, weighed in top pan balance and recorded in g.

3. Germination test

The seeds were surface sterilized with 0.01% (W/V) mercuric chloride for 3 min.

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

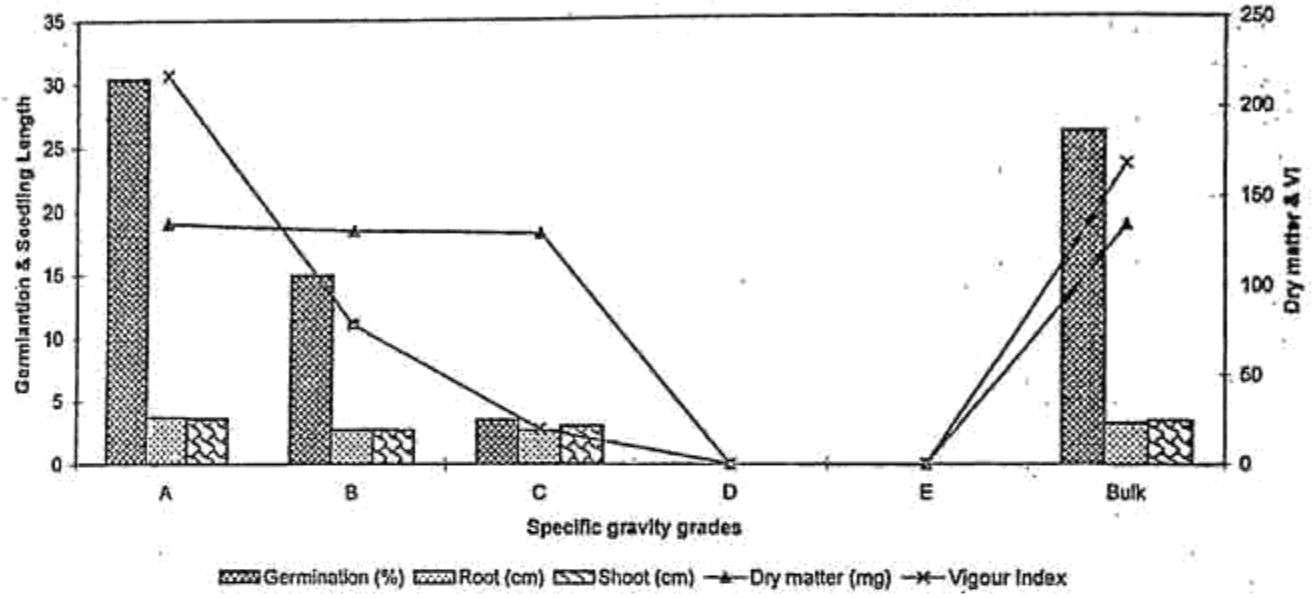
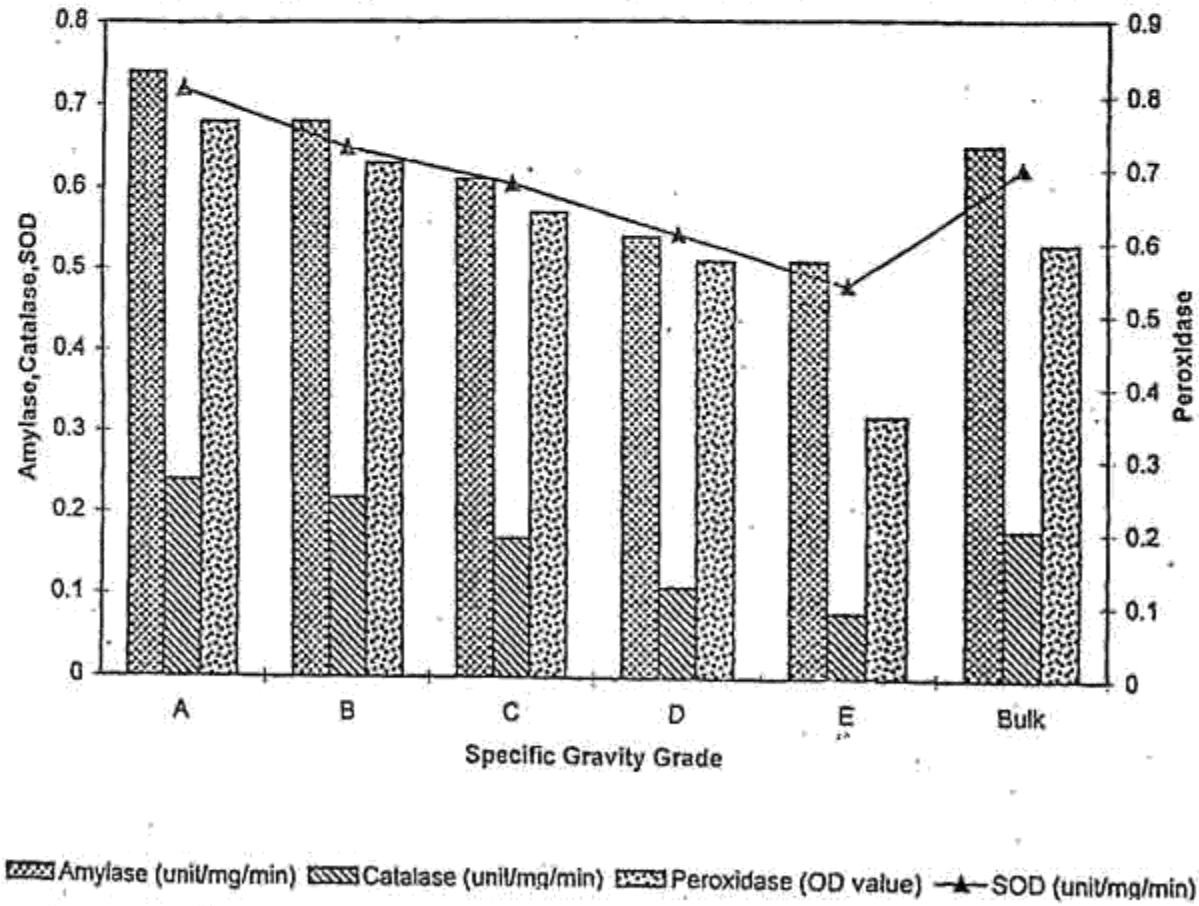


Fig.2. Effect of specific gravity of seeds on enzyme activity of *Casuarina equisetifolia* seed:



washed thoroughly with distilled water and 100 seeds of each grade class were set in roll towel method (ISTA, 1985). Fourteen days after sowing, counts were made and germination was expressed as the percentage of seeds which produced normal seedlings.

After germination count ten random seedlings were measured for their root and shoot length

nd vigour index (Abdul-Baki and Anderson, 973) was derived. Vigour Index = Germination %) x Seedling length (cm). Ten random seedlings ere kept in an oven maintained at 80°C for 4 h. Later the samples were cooled in a esiccator, weighed and expressed in mg/10 eedling.

Amylase

100 mg of seed material after 48 h of mbition was homogenized in 1.8 ml of 0.02 M odium phosphate buffer (pH 6.0). Amylase ctivity was estimated by the amount of starch ydrolysed, determined by the change in the ptical density from time zero to the end of ie reaction at 620 nm (Paul et al. 1970).

Catalase

250 mg of seed material after 48 h of mbition was homogenized in 1.8 ml of 0.066 M odium phosphate buffer (pH 6.8). Catalase ctivity was estimated by the permanganate method Povolotskaya and Sedenka, 1956).

6. Peroxidase

250 mg of seed material after 48 h of imbition was homogenised in 1.8 ml 0.25 M tris buffer (pH 6.0). Peroxidase activity was estimated by the colorimetric method at 402 nm (Malik and Singh, 1980).

7. Superoxide dismutase

250 mg of seed material 48 h of imbition was homogenised in 1.8 ml of sodium phosphate buffer (pH 7.8). The activity of SOD was assayed by measuring it's ability to inhibit the photochemical reduction of nitroblue tetrazolium, at 560nm adopting the method of Beauchampa and Fridorich (1971).

The results were subjected to analysis of variance and tested for significant differences (Panse and Sukhatme, 1967).

Results and Discussion

The results of the experiment revealed that the five grades of seeds viz. A,B,C,D and E differed significantly, showing a gradual decrease in both physiological and biochemical properties with a decrease in seed quality. An assessment of seed filling percentage was done by pricking out the true seeds from the samaras using the dissection needle to estimate the number of filled and empty seeds. The filling percentages recorded for A,B,C,D and E grade were 95, 73, 38, 17 and 10. Bulk ungraded seeds recorded 82.6 per cent filling. The hundred seed weight recorded were 131, 85, 79, 72, 70 and 129 mg for A,B,C,D,E grades and ungraded bulk, respectively (Table 1).

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

Specific gravity class	Seed filling (%)	Hundred 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

Seed germination recorded by A grade seeds was 30 per cent which was followed by B (15.0), C(3) grades and ungraded bulk seeds (26). D and E grades did not germinate at all. The seedling growth and vigour also followed the same trend as that of seed germination (Fig.1). Among the enzymes, amylase alone was recorded in all the seed grades and the magnitude of decrease over A grade was 4.46, 10.44, 56.7, 59.7 and 9.46 per cent, in B,C,D,E grades and bulk seeds, respectively. Catalase, peroxidase and superoxide dismutase were absent in the E grade seeds. The magnitude of decreases of catalase activity was 37.1, 43.0, 75.6 and 40.6 and 70.13 in B,C,D and ungraded bulk, respectively. Similarly for peroxidase 14.28, 41.1, 78.1 and 24.3 per cent decrease were recorded. For superoxide dismutase, it was 22.3, 44.5, 77.8 and 44.5, respectively (Fig.2).

The superiority of the first grade i.e. A grade seeds in seed filling, hundred seed weight and enzyme activity viz., amylase, catalase, peroxidase and superoxide dismutase explains the increased germination and seedling vigour in the A grade seeds. 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 lot. Significant upgrading of *Platanus occidentails* by removal of empty seeds was possible (Bonner and Switzer, 1971). Upgrading of Eucalyptus seeds on specific gravity basis by using water floation method or gravity separator significantly increased the seed weight and germination (Dharmalingam et al. 1973; Khan, 1976). Ponnusamy et al. (1993) advocated density grading of the depulped neem drupes using water to upgrade the seeds for better seedling production.

The results of the present study reveals that close correlation exists between the seed grades and the biochemical potential of the seeds. By adopting seed grading based on its specific gravity, improvement of seed germination and seedling vigour can be obtained because seeds with high specific gravity contain higher enzyme activity also.

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(Received: April 2003; Revised: May 200