

VARIATION IN SEED MASS AS KEY FACTOR IN GERMINATION AND SEEDLING VIGOR IN CASHEW (ANACARDIUM OCCIDENTALE L.)

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INTRODUCTION

ABSTRACT

In order to study the effect of seed size on germination and seedling vigor in cashew (*Anacardium occidentale L.*), an experiment was carried out in the nursery facilities of ICAR-Directorate of Cashew Research, Puttur, Karnataka, India. Variation in seed size showed significant influence on germination percentage, mean germination time, seedling emergence, seedling vigor index, peak value, mean daily germination, germination value, germination energy and germination index. Germination percentage of small, medium and large size seeds are 73.7%, 81.4% and 91.8% respectively. Seed size significantly affected seedling survival with large seeds exhibiting higher survival (60%) compared to medium (55%) and small size seeds (50%). Seedling vigor expressed in terms of shoot and root length, leaf area, leaf number, shoot and root dry weight and total dry matter was significantly affected by seed size. In addition, seedlings arising from large size seeds allocated greater amount of resource to root thus resulted in higher root to shoot ratio. Thus it implies that variation in seed size has significant influence on germination and seedling vigor with large size seeds exhibiting better germination percentage, vigor, growth and survival, higher root to shoot ratio and ability to produce high quality seedlings of cashew.

Cashew (Anacardium occidentale L.) is a tropical evergreen tree, belonging to the family of Anacardiaceae, known for its nuts that are consumed worldwide (Tullo, 2008). It is the gift of nature to the mankind by the virtue of its wide edaphoclimatic adoptability, hardy to adverse climatic conditions as well as pest and diseases, input efficient and foreign exchange earning etc. Cashew was brought to India by Portuguese travelers in 16th century for afforestation and rehabilitation of degraded lands but realizing its importance as hardy crop, precocious bearing habit and good foreign exchange earning crop, it has been adopted as one of the important horticultural crops of India. In view of its vast domestic and industrial applications, cashew enjoys a tremendous demand in the domestic and world market. Despite the importance of cashew as commodity crop with social and economic importance, the crop is threatened with the problems of low yield. Several factors have been mentioned in literatures to be closely related to yield potential of cashew trees. Poor genetic makeup of the existing cashew plantations is one of the reasons for poor and erratic yields. Cashew being highly cross pollinated crop, the plantations raised with nondescript seedlings materials exhibits wide genetic variation with respect to growth, yield and other characters. Many of cashew plantations in the major cashew producing areas of India today produce poor yield probably because of low vegetative vigor of the seedlings used in the establishment of such farms (Ohler, 1979; Martin and Kasuga, 1995; Topper et al., 2001; Aliyu, 2004). Hence, high germination and vigorous seedlings play key role in establishment of good cashew orchards. Therefore, selection for seedling vigor indicators should always be considered in breeding programme to improve overall performance of cashew tree. One of the major production constraints as per cashew is concerned is that most of the plantations are senile and unproductive which has to be replaced with clones of high yielding variety (Huballi, 2009). To boost the cashew production and become self sufficient, there is a need to produce quality planting materials since large planting materials is needed for area expansion and replanting of senile and unproductive cashew orchards. Under seedling stage, sowing of mixed or ungraded seeds give rise to non uniform density of nursery stock. Many times, this results in the production of unhealthy and heterogeneous seedlings of cashew in the nursery. One of the reasons for the heterogeneity in the nursery stock is the high amount of variation in size and weight. Variation in the seedling size could be avoided to a great extent if the seed of uniform grade could be used for nursery sowing. Hence, standardization of nursery techniques for this species is very important and is little known in this species.

Generally, seed germination is controlled by many internal and external factors. Seed size is one among them. Studies of the relationship between seed size and early growth have been reported since early this century (Willenborg *et al*, 2005). Seed size is an important physical indicator of seed quality that affects growth and is frequently related to yield, market grade factors and harvest efficiency. Seed size is an important parameter, which influences the germination, growth and biomass of nursery seedlings (Zareian et al., 2013). Sowing of the mixed seed of a species may result in non-uniform density of seedlings, which may lead to heterogeneity in the vigor and size of the seedlings. Hence, grading plays significant role in maintenance of uniformity in seedlings. The main purpose of seed grading is to understand the better physiological quality of the seed lot (Dar et al., 2002). Grading of seeds based upon their size and weight is a common practice in a majority of plant species as it has been found to regulate the germination and subsequent seedling growth in many species (Ajeesh et al., 2014; Vikas Kumar et al., 2014a; Vidyasagaran et al., 2014b). Seed size is also key evolutionary trait that contributes to early seedling establishment and partitioning of assimilates at reproductive stage (Warringa et al, 1998). Variation in seed size is also associated with traits contributing to competition among plant communities and as well as a determinant for successful establishment of seedlings (Zhang, 1998). Smaller seeds generally germinate faster providing greater competitive advantage especially in early successional stages (Baskin et al, 1998). Nevertheless, larger seeds, although germinating slowly, often have higher percentage of germination than small seeds, being favored in predictable habitats (Mendes-Rodrigues et al, 2011). The weight and size of the seeds are directly related to the amount of nutritional reserves that will be allocated for the initial seedling growth (Mendes-Rodrigues et al, 2011). Large seeds tend to produce more vigorous seedlings when compared to small seeds (Yanlong et al, 2007). Greater amount of stored reserves allow a higher probability of seedling establishment at sites with lower resource availability. However, plants respond to their environmental in such way as to optimize their resource use. Thus, according the resource optimization hypothesis, plants allocate relatively more resource to their root system when nutrient availability increases. In this scenario, it is expected that seedlings originated from larger seeds would present larger root: shoot ratio, as large seeds have more nutritional reserves (Souza et al., 2014).

Inadequate information on the seedlings establishment in terms of effect of seed size on germination and early seedling growth for regeneration programme is a major problem in cashew. The usefulness of seed size as a desirable trait in the regeneration of cashew will provide some information on the biology of the species for its uniform, maximum and rapid propagation in the nursery. Conservation of genetic resources of forest species is very important in the tropical world where ecosystem destruction continues unabated. Therefore a major step aimed at forest ecosystem conservation is species regeneration. Hence, the use of seeds with desirable traits is undoubtedly a major step in the regeneration process. Therefore, the objectives of this study are to determine the effects of seed size on germination indices, seedling vigor and early seedling growth characteristics of cashew under nursery condition.

MATERIALS AND METHODS

Effect of seed size on germination performance and seedling

vigor of cashew (*Anacardium occidentale L*) was studied during April-June, 2016 in the nursery facilities of ICAR-Directorate of Cashew Research (DCR), Puttur, Karnataka, India. The seeds of cashew differing in seed size were collected from approximately 35 trees maintained at experimental plots of DCR, Puttur, for sorting and seedling establishment in the nursery. Seed lots were thoroughly hand mixed to improve the homogeneity and categorized into three seed sizes based on Biodiversity International descriptors: small (S): <5 g; medium (M): 5-7 g and large (L): >7 g. From each class, 50 seeds were randomly selected to record weight, length and breadth (Table 1).

Seeds of each seed size category were sown under nursery conditions in poly bags containing soil, FYM and sand in the ratio of 1:1:1. The bags were placed on a polythene sheet in completely randomized design with seven replications and watered every alternate day. The seeds were regularly monitored in terms of onset of germination and number of seedling emerged over a period of 22 days. The number of days required for onset of germination, seedling emergence, mean time for onset of germination (MGT) and percent seedling emergence for each seed size category were recorded. From these observations, germination percentage (GP), mean daily germination (MDG), peak value of germination (PV), germination value (GV), germination energy (GE) and germination index (GI) were calculated (Czabator, 1962). Germination percentage (GP) was calculated as the number of germinated seeds as a percentage of the total number of the tested seeds is given as; GP = (germinated seeds/total tested seeds) x 100 (Gharieneh et al., 2004). Mean germination time (MGT) is given as; (MGT days) = $S \times Ti \times Ni / S$ Where Ti is the number of days from the beginning of the experiment, Ni the number of seeds germinated per day and S is the total number of seeds germinated (Scott et al, 1984). Germination Index (GI) was calculated by using the following equation: GI $= (G1/1) + (G2/2) + \dots + (Gx / x)$ Where G is the germination day 1, 2..., and x represents the corresponding day of germination (Czabator, 1962). Germination energy (GE) was calculated as the percentage of seed germination obtained at maximum daily germination speed. Peak value (PV) was calculated as the maximum value of cumulative percent germination divided by the days of the test where as mean daily germination (MDG) as the final percent germination divided by the total number of test days (Czabator, 1962). Germination value (GV) is derived by combining peak value and mean daily germination; $PV \times MDG$ (Czabator, 1962). Seedling vigour (SV) is calculated by multiplying the seedling length and germination percentage.

The seedlings were allowed to grow for 60 days after germination for survival and growth studies. After 60 days of growth, seedlings were removed to determine their length and dry mass of shoot and root system. After thorough washing, seedlings were separated into leaves, stems and roots and oven dried for 48 hr. at 65°C to determine dry mass of these seedling biomass components. The relative partionining of seedling biomass was estimated by the ratio of organ to total seedling biomass (g/g) and reported as leaf mass ratio (LMR), stem mass ratio (SMR) and root mass ratio (RMR) (Offiong et al, 2008). Relative growth rate (RGR) was calculated according to Hunt (1982). RGR = $(\ln W2 - \ln W1)/t2 - t1$ where t is the time in days and W1 and W2 are the dry masses per plant in g at t1 and t2, respectively. Root to shoot ratio was calculated as Dry weight of root (g)/dry weight of shoot (g) (Offiong et al, 2008).

The data were analyzed using AGRES statistics. Seed size variations in germination and seedling growth parameters were compared by ANOVA. Correlation analysis was performed to show influence of seed mass on germination and growth parameters. Further, linear regression analysis followed by regression equation was also computed for above relationship.

RESULTS AND DISCUSSION

Seed germination

There were significant differences in germination percentage between the different seed sizes. Large seed size was recorded to have the highest mean germination percentage of 91.87% followed by medium and small seed size with mean germination percentage of 65.91% and 26.08% respectively. Results showed that seed size significantly affected germination period among the different seed sizes. The mean germination time (MGT) for small size seeds was less (11.7) days compared to medium size seeds (12.7 days) and large size seeds (14.05 days) respectively (Fig. 1). On the other hand, large size seeds showed higher and faster seedling emergence than medium and small size seeds and significantly influenced by variation in seed size (Fig. 1). Seed size was positively correlated with germination time ($R^2 = 0.831$, p < 0.01), germination percentage ($R^2 = 0.752$, p<0.01), seedling emergence ($R^2 =$ 0.728, p < 0.01) and seedling vigor index (R² = 0.682, p < 0.05) (Fig. 2). Higher germination percentage (GP), peak value (PV), mean daily germination (MDG), germination value (GV), germination energy (GE) and germination index (GI) were observed in large size seeds and the values were lowest in small size seeds and positively influenced by variation in seed size (Table 2 & 3). Thus, it is evident that variation in seed size clearly influences germination in cashew seedlings in the present study. Small sized seeds germinated faster than medium and large sized seeds under nursery conditions in this study. In general, seed size is directly related to seed coat thickness and inversely related to water absorption (Beninger et al., 1998). The increase in seed size also implies decrease in the surface: volume ratio resulting in lower relative ability to absorb water and initiate the process of germination (Fowler et al., 2000). Thus, small sized seeds have thinner coat and higher relative surface. This seed trait ensures greater permeability in small seeds and require less time for germination (Dolan et al., 1984 and Rai et al., 2014). In the present study, the fact that large sized seeds has thick coat and low surface: volume ratio can justify late germination and variation in germination time between seed size classes of cashew seedlings. Larger sized seeds achieved greater germination percentage than medium and small sized seeds. This greater germinability of large sized seeds may be attributed to the presence of higher amount of carbohydrates and other nutrients than in medium and small sized seeds. This is in agreement with earlier works in tree species (Tripathi and Khan, 1990; Khan and Uma Shankar, 2001). Manonmani et al. (1996) and Gunaga et al. (2007) have recorded higher seed germination and seedling vigor by using bigger sized seeds in Pongamia pinnata and Vateria indica. This trend has also been reported in some multipurpose trees by Negi and Todaria (1997). Ahirwar et al. (2012) also reported that germination percentage was strongly influenced by seed size and that such germination percentage was highest in large seed size compared to the medium and small seed of loblolly pine (Pinustaeda) and Alangiumlamarckii seeds respectively. However, the report of Missanjo et al. (2013) showed that seed sizes of Albizia lebbeck did not have any effect on the germination percentage of the species. The present result indicates that grading of seeds with the aim of enhancing germination percentage in the nursery is very important in cashew. Similarly, this result showed that early germination emergence was favored in large seed size and could be as a result of storage of more food substances in its seed which influenced quicker metabolic activities for faster germination (Bonfil, 1998). In line with this occurrence, Hojjat (2011) reported that large seeds of lentil genotypes showed early seedling emergence compared to medium and smaller size seeds. Thus, seed size emerged as governing characteristics for germination and seedling establishment in cashew seedlings in the present study.

Seedling survival, growth parameters and their relationship

After germination experiments under nursery conditions, emerged seedlings of all seed size classes were allowed to grow for 60 days for seedling survival and seedling establishment studies. Growth parameters in terms of plant height, shoot and root length, shoot and root dry weight, leaf fresh and dry weight, leaf number, leaf area and total plant biomass were recorded after 60 days of growth for all seed size classes. Seedling growth characteristics recorded showed significant variation among three seed size classes. It is evident that the increment in above growth parameters is maximum in large size seeds compared to other seed size classes (Table 4). Bigger sized seeds showed significantly higher seedling height (34.4 cm), shoot length (23.5 cm) and root length (15.3 cm) than those of medium and small size seeds. In agreement to the present study, Nagarajan et al (2006) reported the effect of seed size and seed priming treatment on seedlings growth characteristics and concluded that the large seed size had higher shoot and root length than medium and small seed sizes. Seedling growth measured in terms of plant height (R^2 = 0.766, p<0.01), shoot length ($R^2 = 0.691$, p<0.05), root length ($R^2 = 0.706$, p<0.01), shoot dry matter ($R^2 = 0.541$, p < 0.05), root dry weight ($R^2 = 0.769$, p < 0.01), leaf dry weight $(R^2 = 0.753, p < 0.01)$, total dry matter $(R^2 = 0.84, p < 0.01)$ and leaf number ($R^2 = 0.424$, p < 0.05) at the end of 60 days of germination was significantly affected by seed size (Fig. 3). Seedlings that arose from large size seeds showed better survival (62.2%) than those of medium (54.1%) and small (46%) size seeds after 60 days of growth (Table 4). Seed size also showed significant influence on seedling survival rate $(R^2 = 0.923, p < 0.01)$ with large size seeds showing higher survival than other seed size classes (Fig. 4). Large sized seeds confer advantages to their seedlings for survival and growth due to large reserve of nutritive substances. In this study, we found significant relationship of seed mass with seedling survival and seedling vigor. These findings are in agreement

with Bonfil (1998) who reported positive correlation between seed mass and seedling survival in *Quercus species*. The relationship between seed size and seedling growth characteristics based on regression analysis indicated that seed size promoted seedling vigor (Table 5). The strong influence of seed size in early seedling growth observed in the present study may be the combined effect of larger embryo, high energy reserves, the efficient mobilization of reserves and more rapid emergence of heavier seeds. The more rapid emergence is likely to be due to higher germination percentage and seedling vigor associated with larger reserve tissues and embryos in heavier seeds (Marshall, 1986). The positive relationship between seedling length, total plant biomass and seed size as reported in several other studies support our experimental results (Simmone *et al.*, 2000).

Relationship of seed size with relative growth rate and biomass partitioning parameters

The mean relative growth rate (RGR) values and biomass partitioning parameters *viz.*, root to shoot ratio (R/S), leaf mass ratio (LMR), stem mass ratio (SMR), root mass ratio (RMR) and leaf area ratio (LAR) were significantly influenced by seed size. Seed size showed positive relationship with leaf area (R² = 0.782, p < 0.01) (Fig. 5A), RGR (R² = 0.832, p < 0.01) (Fig. 5C), R/S ratio (R² = 0.472, p < 0.05) (Fig. 5E) and negatively related to LAR (R² = 0.498, p < 0.05) (Fig. 5B) and SMR (R² = 0.487, p < 0.05) (Fig. 5F). Seed size did not show any relationship with LMR (Fig. 5D) and RMR (Fig. 5G).

Another important aspect of the relation between seed and seedling size is related to resource allocation for root or shoot. The larger increase in seedling biomass per unit seed mass resulted from the positive influence of seed size on relative growth rate (RGR) of cashew seedlings. As a result, seed size may have significant influence on initial size of cashew seedlings. The biomass partitioning in terms of root and shoot growth varied with seed size in some tree species (Khurana and Singh, 2000) while it was not in others (Reich, 1994). The high investment in root tissues promotes greater development of root system that reach deeper levels of substrate with more water and nutrients (Canadell et al., 1995) resulting in higher root: shoot ratio in seedlings emerged from large sized seeds as observed in this study. Thus, this biomass allocation between root and shoot could increase the survivorship change of seedlings with poor nutrient reserves.

The study has provided some basic information on germination, seedling vigor and early seedling growth characteristics of cashew. The study indicated that large seed sizes gave the best germination indices in terms of germination percentage, faster seedling emergence and better survival compared to the medium and small seed sizes. It was also shown that large seed size had the highest values for all the growth parameters measured followed by the medium seed size while the small seed size had the lowest values. The study significantly showed that seed size is very important in the germination, early seedling growth, seedling vigor index, biomass production and seedling establishment in tropical forest tree species like cashew. The result from this study is vital to horticulturists and can provide plausible options for regenerating the species for conservation and domestication purposes. The large seed size is recommended, as it would ensure rapid maximum and uniform germination as well as promote early maturity of cashew seedlings which is pivotal to high quality seedling production in the nursery.

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