



Germination and seedling establishment in cashew (*Anacardium occidentale* L.): An interaction between seed size, relative growth rate and seedling biomass

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Abstract

Seeds of cashew were used to determine the effect of seed mass (5.2 to 7.8 g) on germination, seedling emergence and growth under nursery conditions. Germination percentage and germination time showed significant correlation with seed mass. Large sized seeds had higher germination percentage (81.6%) and produced more vigorous seedlings. Per cent seedling emergence was related to seed mass with large sized seeds exhibited faster emergence. Seed mass significantly affected seedling survival and survival rate was high in seedlings arising from large sized seeds (62.9%). Seedling vigor expressed in terms of shoot and root length, leaf number, leaf area and total dry matter was significantly affected by seed mass. Seedlings that emerged from large sized seeds showed better growth and produced heavier seedlings as compared to medium sized seeds. RGR showed significant variation (0.152 to $0.240 \text{ g g}^{-1} \text{ day}^{-1}$) among two seed size classes positively correlated with seed mass, leaf area (LA), unit leaf rate per unit leaf area (ULR_M), root to shoot ratio (R/S) and root mass ratio (RMR) and negatively with stem mass ratio (SMR). The study concluded that the seed mass and RGR have influence on seedling growth and success of seedling establishment in cashew.

Keywords: Cashew, germination, RGR, seed mass, seedling survival, seedling vigor

Introduction

Seed germination and seedling establishment in plants are influenced by a series of events which in turn govern the distribution, abundance and relative performance of the species. In fact, variations in several seedling characteristics and seed germination are also known to influence the relative abundance of plant species and type of climatic conditions within where a seedling can establish in a plant community (Grubb, 1976; 1977). Seed size, an important plant trait of reproductive strategy, affects the process of seed germination and further seedling establishment (Grubb, 1996). Variations in seed size also have strong influence on germination time (Murali, 1997), germination percentage (Mölken *et al.*, 2005) and seedling vigor (Yanlong *et al.*, 2007) which, determine the distribution of plant species across different plant habitats (Silveira *et al.*, 2012). Generally, plants

with large seed size confers competitive advantages with more rapid emergence, larger initial seedling size and better survival capacity under varied environmental conditions (Gross and Werner, 1982; Black, 1958; Righter, 1945 and Bonner, 1987). Studies by Primack (1987) demonstrated that the initial size and weight of seeds are directly related to nutritional reserve content which ultimately influences the initial seedling growth and establishment. Hence, large sized seeds produce more vigorous seedlings than small sized seeds (Yanlong *et al.*, 2007). Thus, seed size variability is an important factor of plant growth that influences the various stages of plant performance and establishment such as seed germination, seedling recruitment and also its relative abundance and performance under different climatic conditions (Leishman, 2001; Rego *et al.*, 2007). Apart from variation in seed size, other determinants such as

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difference in length of growing period, emergence rate, vigor index and relative growth rate also affect seedling emergence, seedling establishment and biomass production.

Grime and Hunt (1975) studied inter specific variation in RGR based on three distinguished groups of plant species namely species from ruderal habitats or places with intensive competition, species with high potential RGR and species growing in adverse environment with low potential RGR. Their studies have also indicated the ecological importance of high RGR which may result in fast growth for species growing in competition situations and rapid completion of plant life cycle especially for ruderals. Studies on variations in RGR and its components on wide range of species being representative of varied nutrient condition of natural habitats, life forms and taxonomic families have shown the differential relationships of RGR with seed size being negatively (Shipley and Peters, 1990) or positively correlated (Meerts, 1995). Among growth parameters, leaf area (LA) also correlates negatively with carbon assimilation rate in plant species (Evans, 1993).

Cashew (*Anacardium occidentale* L.) is an important tropical nut crop of social and economic importance worldwide. World's total area under the cultivation of cashew is around 35100 km² with India sharing 20 and 16 per cent of cashew area and production globally. Cashew is primarily grown for its kernel being highly nutritive and low cholesterol content. Other commercial and economic benefits of cashew include value added products such as juice, wine, vinegar, jam, pickle and cashew nut shell liquid (CNSL) for industrial uses. Despite the importance of cashew as commodity crop with social and economic importance, the crop is threatened with the problems of low yield.

High germination and vigorous seedlings are important factors in establishment of good cashew orchards. Therefore, selection for seedling vigor indicators may be considered as one of the key factors in any breeding programme to improve crop performance. Many of the cashew plantations in major producing countries like India, Brazil and Tanzania today produce poor yield probably because of low vegetative vigor of the seedlings used in the establishment of such farms. One of the major production constraints in the country is that

most of the plantations are senile and unproductive which has to be replaced with clones of high yielding variety (Huballi, 2009). Sowing of mixed seeds gives rise to non-uniform density of nursery stock. Many times, this results in the production of unhealthy and heterogeneous seedlings 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. Studies by Ajeesh *et al.* (2014) have shown the benefits of grading of seeds based on size and weight which in turn has regulative influence on seed germination and growth of the seedling growth in many plant species.

In this background, the present study was undertaken to evaluate the influence of seed size on seed germination and initial seedling establishment under nursery conditions in cashew. The relationships between seed mass and RGR and their effects on seedling growth and development were analyzed in this study. The following hypotheses were addressed to evaluate the combined influence of seed size, RGR and its components on germination behavior and seedling performance in cashew, i) to evaluate whether large sized seeds have higher germination percentage and produce more vigorous seedlings, ii) to investigate the extent of differences in the patterns of seedling emergence, iii) to investigate whether RGR is negatively or positively correlated with variations in seed size and iv) to evaluate the extent and pattern of variation of RGR and its components within this species.

Materials and methods

Seed source

Cashew seeds were collected from thirty randomly selected trees at experimental plots of Directorate of Cashew Research (DCR), Puttur, Karnataka. The seeds were sundried for 20 days and were then stored at room temperature till the initiation of experiment. Seed mass ranging from 5.2 to 7.8 g were selected for the present study. Mean seed mass was determined by weighing individual healthy seeds (n= 50). The healthy seeds were later classified into two categories based on their seed mass: Medium/intermediate (5-7 g) and high (>7 g), respectively to evaluate the combined

influence of seed mass and RGR on seed germination and seedling growth. This classification is based on IBPGR descriptors of cashew germplasm accession (IBPGR, 1986).

Germination experiment in nursery

The influence of seed mass on seed germination was investigated using one hundred and eighty healthy sound seeds from each of two seed size categories under nursery conditions. After 24 hrs of soaking in de-ionized water, the seeds were sown directly in polythene bags (one seed per bag) containing potting mixture of sand, soil and farm yard manure in the ratio of 1:1:1. The experiment was planned in completely randomized design with seven replications. The polythene bags were watered every alternate days and number of seeds germinated were recorded daily till the germination has completely stopped (up to 28 days). Germination percentage and mean germination time (MGT) were calculated on the basis of total number of seeds sown in the nursery.

Seedling emergence, growth and morphology

The influence of seed mass on seedling emergence and seedling vigor were evaluated from seed germination experiments in the nursery. The healthy seedlings were allowed to grow for four months to evaluate the effect of seed mass on seedling growth and morphology. After four months of growth, seedlings were removed to determine their length and other growth parameters such as fresh and dry weight of shoot and root. The seedlings were also separated into leaves, stems and roots and dried in oven for 48 h at 65°C to determine the biomass of seedlings. The seedling biomass partitioning was calculated by the ratio of individual plant organ (leaf, stem and root) to total seedling biomass (g g^{-1}) and indicated as leaf mass ratio (LMR), stem mass ratio (SMR) and root mass ratio (RMR). The growth components namely relative growth rate (RGR, g dry mass increment per day per g biomass) and unit leaf rates (plant dry mass increment per day per unit leaf area, ULR_A or per unit leaf mass, ULR_M) were calculated according to Hunt (1982).

Data analysis

The data on seed germination, seedling emergence and seedling growth parameters were

subjected to analysis of variance (ANOVA). The associations between seed mass, RGR and seedling biomass were analyzed by linear regression analysis. Differences of RGR and its components among varieties were evaluated by means of ANOVA. The associated correlations of characters such as seed mass, RGR, ULR_A , ULR_M , LAR, LMR, SLA, MAL, SMR, RMR and DML were estimated by Pearson's correlation co-efficient. All statistical analysis was performed with MSTATC. All values reported in the present study are mean values.

Results and discussion

Seed mass and germination

The overall seed germination percentage of two seed size categories of cashew reached 78.4 per cent. Seed germination under nursery conditions began 12 days after sowing and continued up to 28th day till the germination ceased to emerge. The variation in seed size has strong influence on seed germination percentage with large sized seeds having higher germination percentage (81.6%) than medium sized seeds (71.9%). In contrast, medium sized seeds showed faster seed germination with mean average time of 12 days compared to large sized seeds which took 14 days for initial seed germination (Table 1). Variations in seed mass showed significant positive correlation with both germination percentage ($R^2 = 0.732$, $p < 0.01$) and mean germination time ($R^2 = 0.765$, $p < 0.01$) (Fig. 1).

There was wider inter-specific variability in seed mass among tropical tree species (Khan *et al.*, 1999; 2002). Variation in seed mass was found to influence germination in cashew varieties. In the present study, medium sized seeds showed faster germination compared to large sized seeds.

Table 1. Seed weight (NWT), percentage germination (GP) and mean germination time (MGT) after 28 days of two seed size classes of cashew. For seed weights n=50 for each size class: medium (M) and large (L)

| Seed class | NWT(g) | GP (%) | MGT (days) |
|---------------|--------|--------|------------|
| Medium (5-7g) | 5.9 | 71.9 | 12.6 |
| Large (>7g) | 7.6 | 81.7 | 14.1 |
| SE (\pm) | 0.3 | 0.2 | 0.3 |
| CD (0.05) | 0.6 | 0.5 | 0.6 |
| CV (%) | 7.3 | 0.6 | 3.6 |

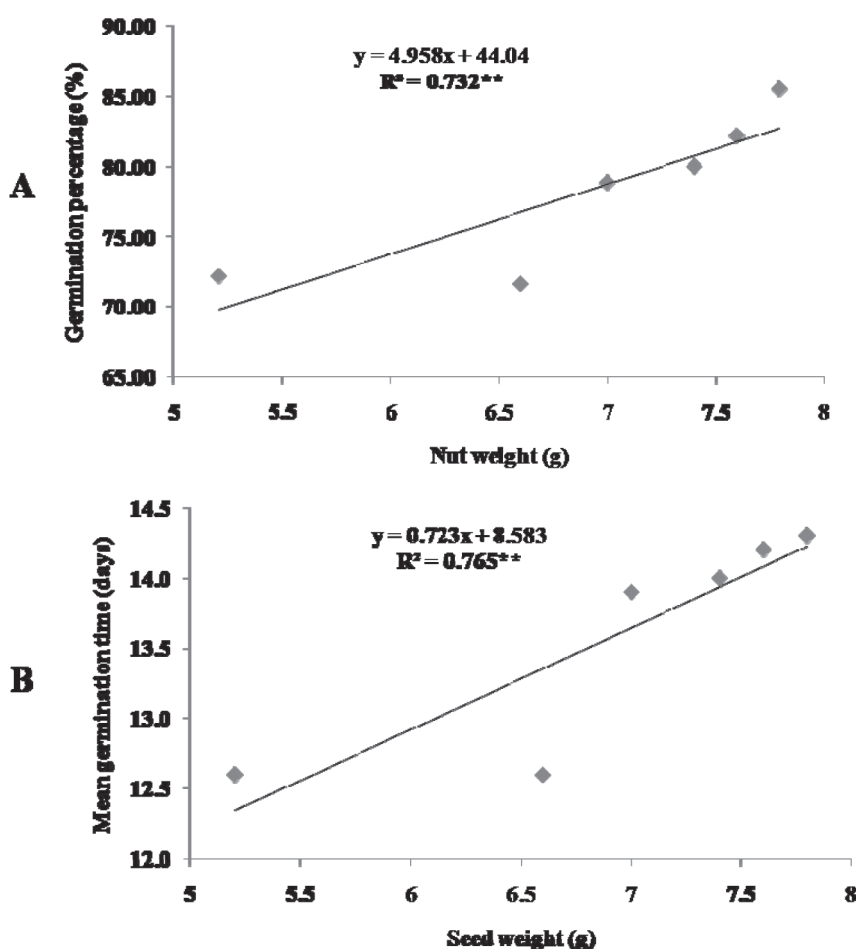


Fig. 1. Relationship between seed weight with (A) germination percentage and (B) mean germination time for two seed size classes of cashew varieties. Germination data (mean values) are from nursery trials after 28 days of germination

Such studies are in consistent with studies by Beninger *et al.* (1998) who reported direct relationship of seed size with seed coat thickness and inverse associations with water absorption; the inverse relationship of increase in seed size with surface to volume ratio resulting in lower ability to absorb water and to initiate the seed germination process (Fowler and Bianchetti, 2000) and also higher permeability of small sized seeds due to thinner seed coat and higher surface resulting in faster germination (Dolan, 1984). Murali (1997) and Khan *et al.* (1999) also reported similar results in other tropical tree species. However, studies by Khan *et al.* (2002) and Tripathi and Khan (1990) reported contradictory results indicating faster initial seed germination by large sized seeds

compared to small sized seeds. In the present study, large sized seeds showed slow initial germination which may be attributed to thicker seed coat and low surface to volume ratio resulting in variability in mean germination time between two seed size categories of cashew varieties. In this study, larger sized seeds showed higher seed germination percentage compared to medium sized seeds and this higher germination may be attributed to larger nutritive reserves of these seeds. This study is in concurrent with earlier reports in several tree species by Tripathi and Khan (1990) and Khan *et al.* (1999).

Seedling emergence and growth

The healthy seedlings emerging from medium and large sized seeds were used to compare the

seedling emergence pattern in cashew varieties. Overall, the seedling emergence started 10 to 13 days after sowing in both seed size classes under nursery conditions. Large sized seeds resulted in faster seedling emergence achieving 50 per cent maximum emergence after 15 days of seed germination whereas medium sized seeds took 20 to 25 days to achieve 50 per cent germination (Fig. 2A). Variations in seed mass showed significant positive correlation with seedling emergence rate ($R^2 = 0.76$, $p < 0.01$). The per cent emergence was also considerably higher

in seedlings emerged from large sized seeds than seedlings from medium sized seeds (Fig. 2B).

Variations in seed mass also significantly influenced the seedling growth parameters measured in terms of seedling height and seedling weight (Fig. 3). The initial seedling height and seedling weight measured after 20 days of germination showed positive relationship with seed mass ($R^2 = 0.771$ and $R^2 = 0.836$, $p < 0.05$) (Fig. 3a and 3b) and after 120 days of growth, the final seedling height and seedling weight were also

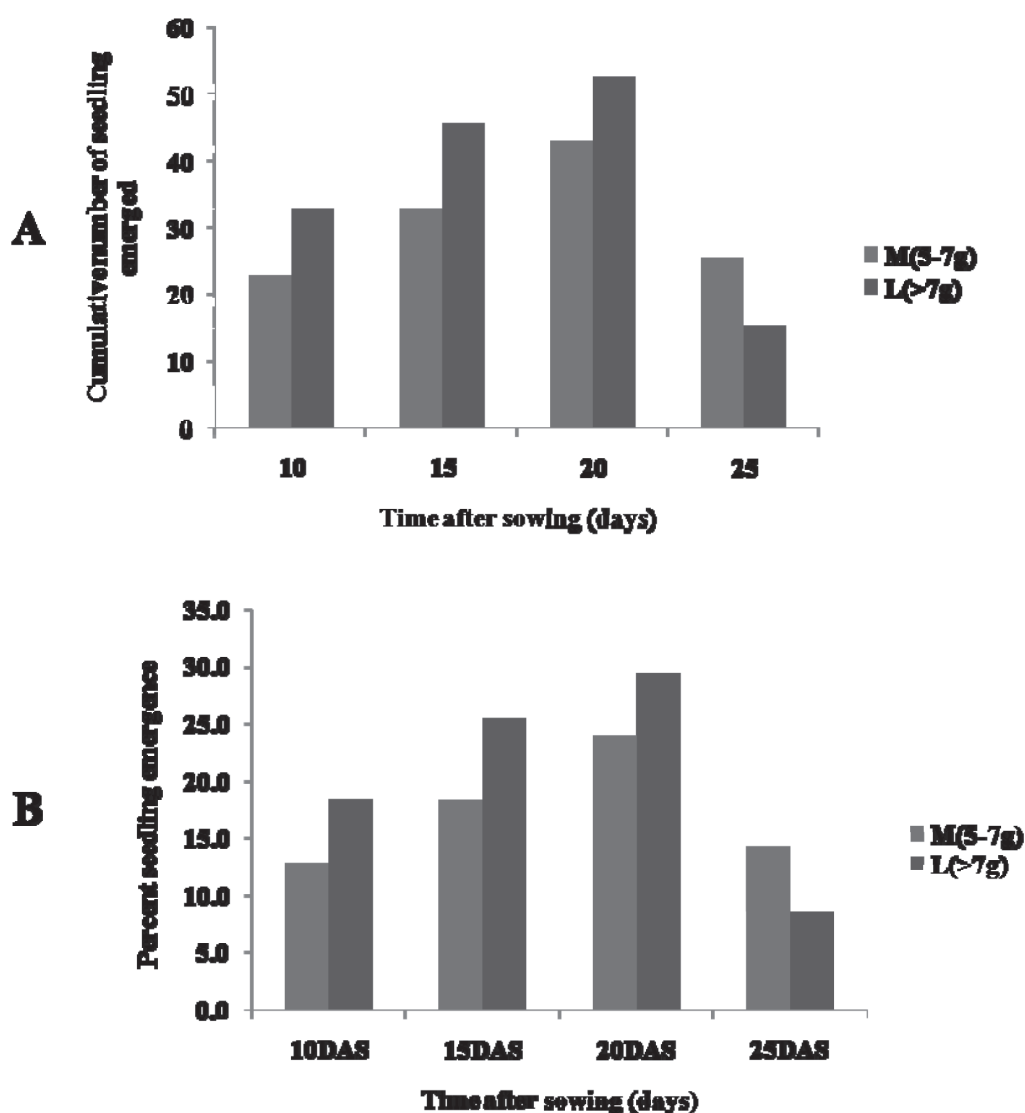


Fig. 2. Patterns of seedling emergence of cashew seedlings. (A) Cumulative number of seedling emerged over 25 days after germination; (B) Mean total percentage emergence of seedlings over 25 days after germination

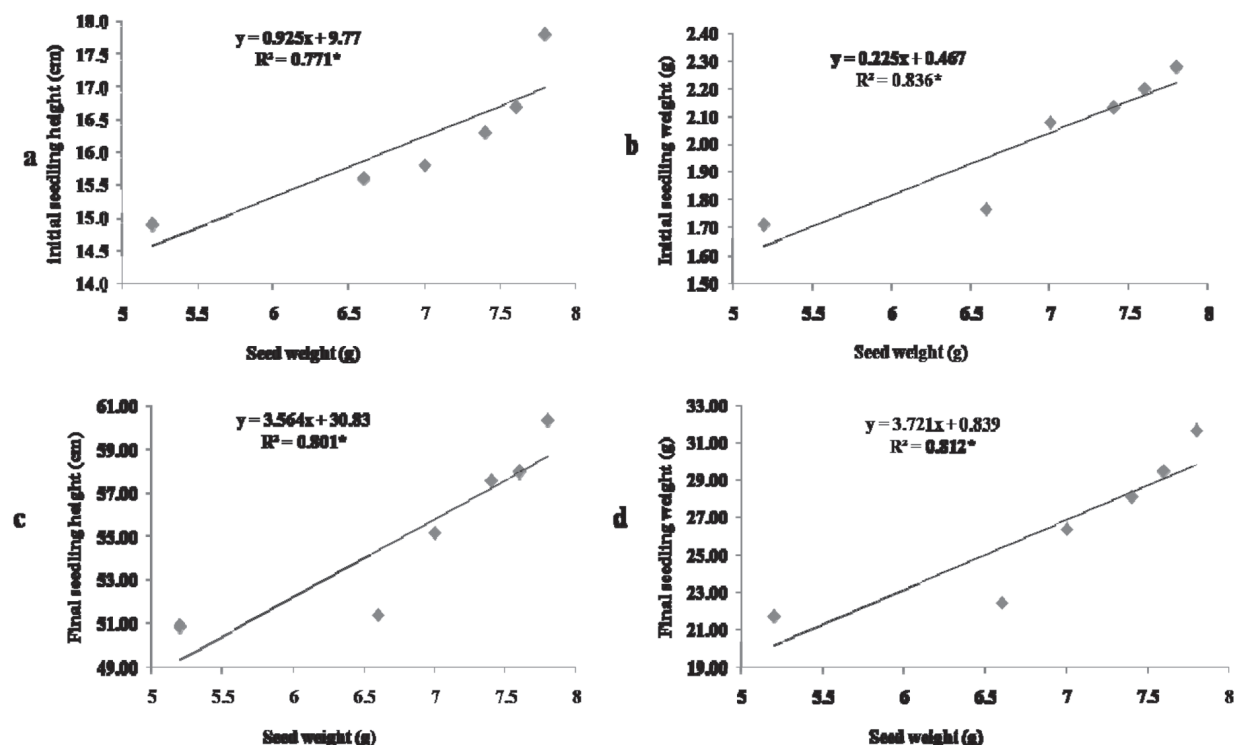


Fig. 3. Relationship between seed weight with (a) average initial seedling height and average initial seedling weight; (b) average final seedling height and average final seedling weight for two seed size classes of cashew varieties. Initial seedling height and weight were determined from newly emerged seedlings (20 days after germination) and final seedling height and weight were determined 120 days after sowing

positively correlated with variations in seed mass ($R^2 = 0.801$, $R^2 = 0.812$, $p < 0.05$) (Fig. 3c and 3d).

In the present study, seedlings that emerged from large sized seeds resulted in early seedling establishment and tend to be heavier than seedlings from medium sized seeds. This may be attributed to several reasons namely the combined effect of larger embryo, better seedling vigor associated with larger reserve tissues embryo, efficient mobilization of reserves and more rapid emergence of larger seeds (Mitchell, 1939).

Seedling survival and growth

Seedlings from large sized seeds showed better survival (63%) than those from medium sized seeds (56%) (Table 2). The seedling growth parameters and root characteristics were also significantly influenced by variations in seed mass (Table 2 and Fig. 4). Among seedling growth parameters, seed mass showed significant positive linear associations with leaf, shoot, root and total seedling dry mass (Fig. 4). In the present study, large sized seeds

produced healthy and vigorous seedlings than those from medium sized seeds and also showed significant positive associations of seed mass with seedling survival and seedling vigor. These results are in consistent with studies by Bonfil (1998) who reported positive relationship between seed mass and seedling survival in *Quercus* species.

Seedling size and relative growth rate (RGR)

The growth parameters namely RGR and its components of two seed size categories were formulated to understand seed mass and seedling growth relationship. Mean RGR value ranged from $0.152 \text{ g g}^{-1} \text{ day}^{-1}$ to $0.227 \text{ g g}^{-1} \text{ day}^{-1}$. In average, seedlings from larger seeds showed higher values of RGR, LA, NAR, URL_A and URL_M and R/S ratio and lower values of LAR, LMR and SMR compared to seedlings from medium seeds (Table 3).

In this study, the biomass partitioning in terms of shoot and root growth varied significantly with variations in seed mass and seedlings from large sized seeds also resulted in higher root to shoot ratio.

Table 2. Seedling attributes of cashew derived from two seed size classes after 120 days of growth

| Seed class | PHT | SL | RL | LFW | LDW | LN | LA | RFW | FDW | RTH | BFW | BDW | SFW | SDW | SSR | SVI |
|---------------|------|------|------|------|------|-----|-------|------|-----|------|------|------|------|------|------|------|
| Medium (5-7g) | 51.1 | 46.3 | 25.2 | 1.41 | 1.17 | 25 | 91.2 | 13.8 | 2.8 | 0.56 | 53.0 | 22.1 | 52.0 | 21.5 | 56.0 | 13.3 |
| Large (>7g) | 57.8 | 55.2 | 28.7 | 1.73 | 1.38 | 33 | 107.6 | 16.3 | 4.7 | 0.91 | 68.1 | 28.9 | 67.0 | 28.0 | 62.9 | 16.0 |
| SE (±) | 0.6 | 0.4 | 0.4 | 0.0 | 0.0 | 0.9 | 1.1 | 0.2 | 0.1 | 0.0 | 0.2 | 0.5 | 0.4 | 0.3 | 0.6 | 0.1 |
| CD (0.05) | 1.3 | 0.8 | 0.8 | 0.0 | 0.0 | 1.9 | 2.3 | 0.5 | 0.2 | 0.0 | 0.5 | 1.1 | 1.0 | 0.6 | 1.4 | 0.3 |
| CV (%) | 2.1 | 1.3 | 2.6 | 1.5 | 2.3 | 5.6 | 2.0 | 2.6 | 4.3 | 5.3 | 0.7 | 3.6 | 1.4 | 2.1 | 2.0 | 1.8 |

PHT denotes plant height (cm); SL: shoot length (cm); RL: root length (cm); RTH: root thickness (cm); LN: leaf number; LFW: leaf fresh weight (g); LDW: leaf dry weight (g); SFW: shoot fresh weight (g); RFW: root fresh weight (g); RDW: root dry weight (g); BFW: biomass fresh weight (g); BDW: biomass dry weight (g); SSR: seedling survival rate (%) and SVI: seedling vigor index (%)

This is in agreement with studies by Khurana and Singh (2000) who reported similar significant relationships between seed mass with shoot and root growth in tree species while contradictory results were also reported by Reich *et al.* (1994) indicating no relationships between them. The higher

root:shoot ratio in seedlings from larger seeds observed in the present study is in consistent with studies by Canadell and Zedler, (1995) who reported the similar results which may be attributed due to high investment in root tissues promoting better development of root system and also better ability

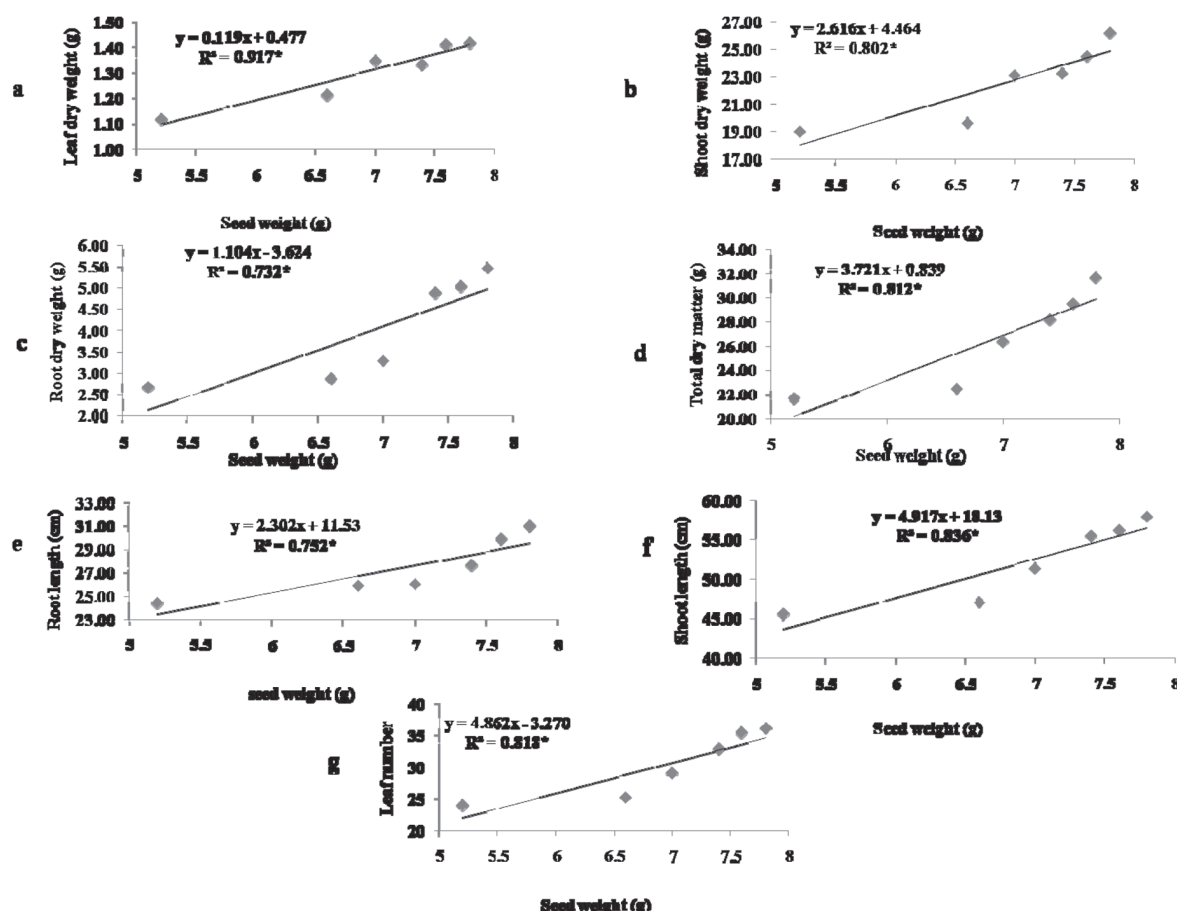


Fig. 4. The relationship of seed mass with (a) mean leaf dry mass, (b) shoot dry mass, (c) root dry mass, (d) total seedling dry mass, (e) root length, (f) shoot length and (g) leaf number. Linear regression equations and coefficients of determination (r^2) are presented for relationships between seed weight and seedling attributes. *, ** indicate significance at $p < 0.05$ and 0.01 level

Table 3. Biomass production and partitioning in cashew seedlings

| Seed class | SLA | SLW | LWR | LAR | RGR | NAR | R/S | LMR | RMR | SMR | URL _A | URL _M |
|---------------|------|------|------|-----|------|------|------|-------|------|------|------------------|------------------|
| Medium (5-7g) | 78.8 | 12.8 | 0.05 | 4.1 | 0.15 | 0.21 | 0.13 | 0.07 | 0.16 | 1.17 | 0.24 | 19.0 |
| Large (>7g) | 78.1 | 12.9 | 0.05 | 3.7 | 0.21 | 0.24 | 0.15 | 0.08 | 0.21 | 1.25 | 0.27 | 21.0 |
| SE (±) | 1.7 | 0.4 | 0.00 | 0.1 | 0.00 | 0.00 | 0.00 | 0.001 | 0.00 | 0.01 | 0.00 | 0.4 |
| CD (0.05) | 3.7 | 0.8 | 0.00 | 0.2 | 0.00 | 0.00 | 0.01 | 0.00 | 0.01 | 0.02 | 0.01 | 0.8 |
| CV (%) | 4.1 | 5.5 | 4.24 | 4.1 | 1.50 | 1.71 | 4.24 | 2.83 | 4.26 | 1.12 | 2.37 | 3.5 |

LA denotes leaf area (cm²); SLA: specific leaf area cm²g⁻¹; SLW: specific leaf weight (mg cm⁻²); LMR: leaf mass ratio g g⁻¹; RGR: relative growth rate gg⁻¹day⁻¹; NAR: net assimilation rate g cm⁻²day⁻¹; R/S: root to shoot ratio; SMR: shoot mass ratio and RMR: root mass ratio, URL_A: unit leaf rate per unit leaf area and URL_M: unit leaf rate per unit leaf dry mass

to reach deeper layers to penetrate more water and nutrients. Thus, the biomass partitioning between root to shoot growth can seen as an important plant trait that could enhance the survivorship change of seedlings under varied environmental hazards especially with poor nutrient reserves.

Relationships between RGR and its components with seed mass

Among growth parameters, RGR (Fig. 5A), NAR (Fig. 5B), leaf area (Fig. 5C) and RMR (Fig. 5F) were positively correlated with seed mass. The SMR (Fig. 5E) showed negative relationship

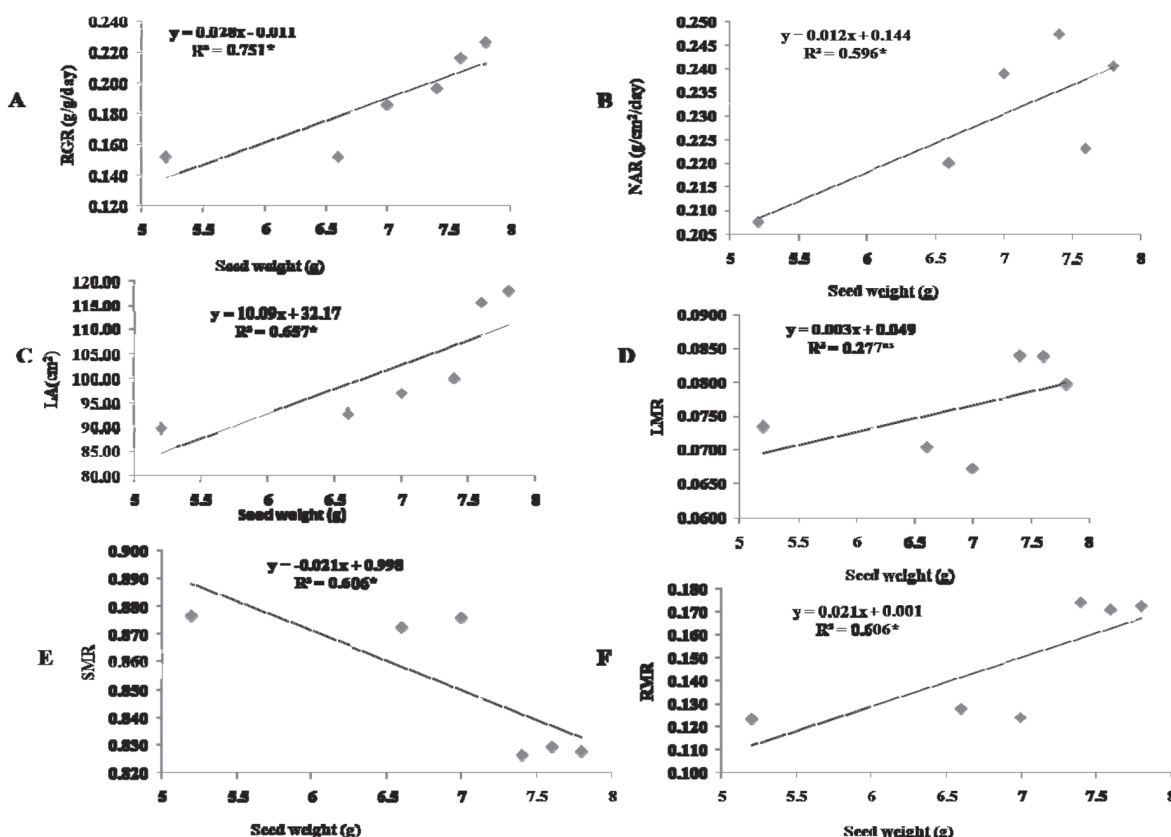


Fig. 5. Associations between seed dry weight with relative growth rate and its components among cashew varieties. (A) Correlations between seed weight with relative growth rate (RGR), (B) with net assimilation rate (NAR), (C) leaf area (LA), (D) leaf mass ratio (LMR), (E) stem mass ratio (SMR) and (F) root mass ratio (RMR). The level of significance is as follows: * $P < 0.05$; ** $P < 0.01$. Linear regression equations and coefficients of determination (r^2) are presented for relationships between seed weight and relative growth rate and also its components

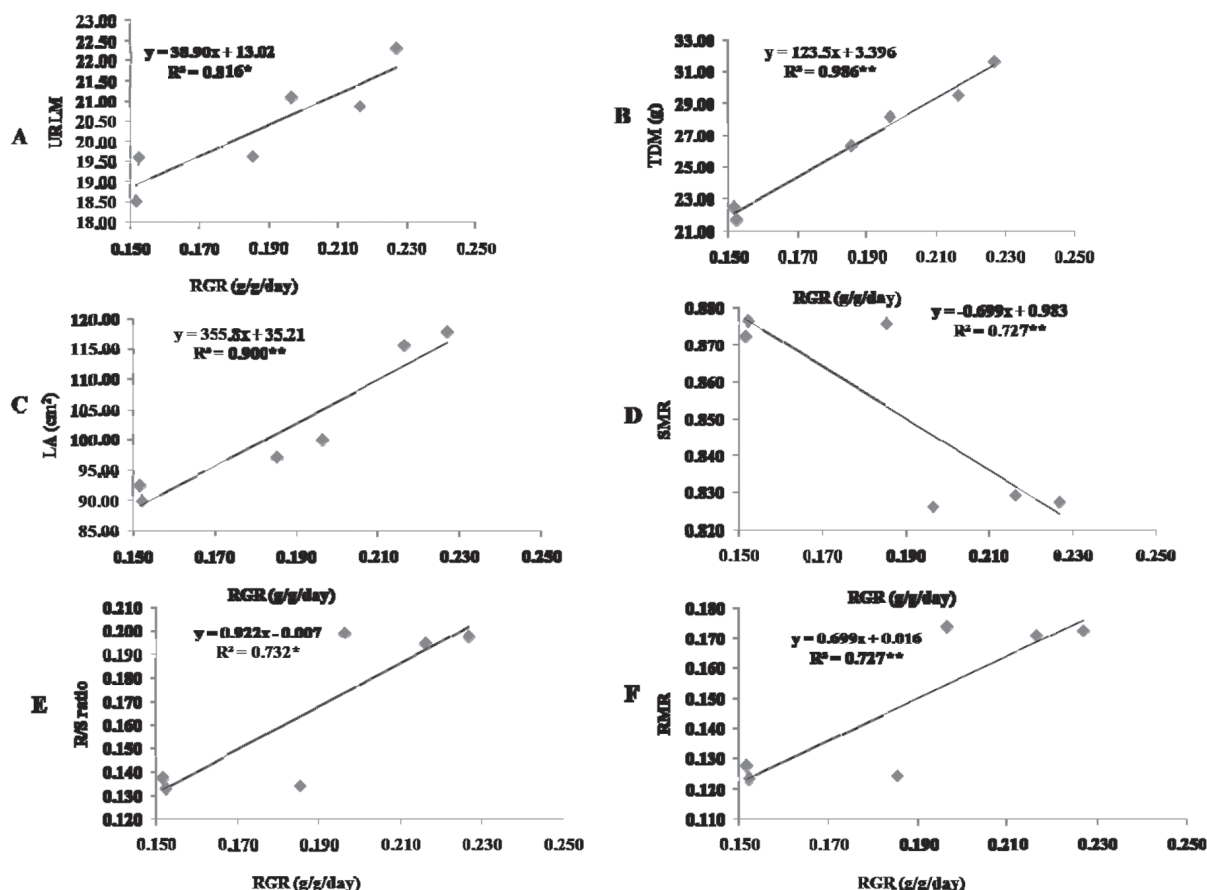


Fig. 6. The relationships between relative growth rate with its components among cashew varieties. (A). Associations between RGR and unit leaf rate per unit leaf dry mass (ULR_M), (B) RGR with total dry matter (TDM), (C) RGR with leaf area (LA), (D) RGR with stem mass ratio (SMR), (E) RGR with root/shoot ratio (R/S) and (F) RGR with root mass ratio (RMR). The level of significance is as follows: * $P < 0.05$; ** $P < 0.01$. Linear regression equations and coefficients of determination (r^2) are presented for relationships between RGR and its components

while LMR (Fig. 5D) did not show any associations with seed mass. Variations in seed mass showed significant positive associations with leaf area ($R^2 = 0.657$, $p < 0.05$), RGR ($R^2 = 0.751$, $p < 0.05$), NAR ($R^2 = 0.596$, $p < 0.05$) and RMR ($R^2 = 0.606$, $p < 0.05$). Among growth components, ULR_M ($R^2 = 0.816$, $p < 0.05$) (Fig. 6A), total dry matter (TDM) ($R^2 = 0.986$, $p < 0.01$), LA ($R^2 = 0.900$, $P < 0.05$) (Fig. 6C), R/S ratio ($R^2 = 0.732$, $p < 0.05$) (Fig. 6E) and RMR ($R^2 = 0.727$, $p < 0.01$) (Fig. 6F) were positively correlated with RGR. The SMR ($R^2 = -0.727$, $p < 0.01$) (Fig. 6D) showed negative associations while specific leaf area (SLA) and LMR showed no associations with RGR (data not shown).

The resource allocation between root to shoot growth significantly influence seed and seedling size.

In this study, the larger increase in seedling biomass per unit seed mass may be attributed to significant positive influence of seed mass on relative growth rate and its components. Thus, variations in seed mass may impart significant influence on initial seedling size of cashew varieties. The positive association between seed mass and RGR was observed in this study. However, several contradictory results were also reported wherein seed mass and RGR were negatively correlated (Shipley and Peters, 1990) while it showed no relationship between them (Twamley, 1967; Choe *et al.*, 1988). Studies by Maranon and Grubb (1993) in 27 annual species support the present study data where the positive associations between seed mass and RGR may be attributed due to significant relationships between leaf area and seed mass.

Conclusions

It is concluded that seedlings from large sized seeds resulted in higher germination percentage, rapid seedling emergence, larger initial seedling size and greater biomass allocation in root to shoot growth compared to those from medium sized seeds in cashew varieties. Cashew varieties showed wide variation in seedling growth parameters. The study also exhibited positive relationship between seed mass and RGR. Thus, this study highlights the significant influence of variations in seed mass on successful seedling establishment by affecting several other factors namely relationship between seed or seedling size, germination condition, emergence time and RGR.

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