Assessment of seed germination and seedling growth of *Vachellia sieberiana* Under different soil moisture regimes

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Abstract

This study aimed at investigating causes and prescribe appropriate seed treatments of *Vachellia sieberiana*. Ten trees were selected in Songa pastures and surveyed for the presence of seeds and saplings under and nearby the canopy. Seeds were collected from tree floor and were treated using hot (500°C) wire scarification, roasting for 30, 40, 50, 60 and 90 minutes and soaking in cold and hot water for 24 hours. Seed germination was tested on sound seeds from February-May, 2010 and seedlings assessed for growth and survival under different watering regimes, by applying 60ml of water/seedling daily, once or twice a week. Seedlings were raised as bare-rooted in open nursery or as potted in a greenhouse. The effect of watering regime on seedling growth and survival, height, number of leaves, leaflets and thorns, root collar diameter, root length and total biomass were observed during the experimental period. More than 50% of *V. sieberiana* seed on the canopy flow retained viability. Seed germination was sporadic and differed very significantly between treatments. Scarification gave the highest germination rate followed by soaking in hot and cold water. Soaking in hot water gave a more uniform germination and is thus recommended for seed treatment in this species. Growth in all traits except root length increased significantly with watering frequency. High root length was positively correlated with fine root density and may indicate ecological strategy to increase water uptake by *V. sieberiana*.

Key words: Seed dormancy, survival strategy, early seedling growth, root length, fine root

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Introduction

Vachellia L. is a relatively new genus after the re-typification of the genus Acacia. The genus Acacia was the largest and most diverse of all, with the number of species estimated at 129 in Africa alone (Gourlay, 1995; Barnes 2001) and 1450 species worldwide (Lewis et al., 2005). The species of the genus were naturally found in Africa, Australia, southern America and Asia (Murphy, 2008). The change of the genus name Acacia was ratified at the XVIII IBC held in Melbourne in 2011 and African acacias were put under genus Vachellia or Senegalia (Lewis 2017).

The genus Acacia has been ignored in reforestation programs, probably because it is not used for timber production. Tree planting in Rwanda is currently done on mostly very marginal land of low soil pH and low fertility (Kerkhof, 1990). Most of the highlands, hills and mountains have been planted with exotics such as Eucalyptus, Pinus and Callitris species which in most cases have accelerated the processes of land degradation and problems of water pollution and siltation (Nduwamungu et al., 2007). This has raised a growing concern that has resulted in revising reforestation policies. For instance, it is prohibited to plant Eucalyptus species in sensitive areas such as marshlands, catchment areas, river banks and lake shores (RoR, 2010).

African vachellias yield a wide range of tangible and intangible products useful to mankind and to wildlife (Maundu and Tegnas, 2005; Dharani, 2002). Yet they have never been deliberately raised as plantations or as components of agroforestry systems in many countries. In Rwanda, Vachellia species grow naturally in all agro-ecological zones (Bloesch et al. 2009; Nduwayezu et al., 2009) but most of the Vachellia trees are mainly hosted in the eastern Rwanda which is characterised by low and sporadic rains. The Akagera National Park and a few remnant forests in the zone are the main host, where trees are seen on degraded lands and on farmlands in the periphery of the park. vachellias are well known by the local community in Rwanda since each of them has a vernacular name.

Vachellia species seed are known to have hard seed coats which interfere with water imbibition which may delay and reduce seed germination (Abdel-Dafai, 1977; Teketay, 1998; Sya et al., 2001; Al-Khateeb, 2005). It is speculated that germination of Vachellia seed may be enhanced when seed coats are damaged due to infection or other physical factors in which case physical dormancy is eliminated (Lamprey et al., 1974; Ahmed, 2008). In fact, infestation with Bruchid beetles has been reported to show such promotive effects on early germination and establishment of Vachellia sieberiana (Mucunguzi, 1995). Certain treatment methods are needed to break the dormancy for the Vachellia seeds to germinate. The commonly used method is by treating seeds with concentrated sulphuric acid (Warrag and Eltigan, 2005). Many studies have confirmed a dormancy relief in legume seeds after fire (Bradstock and Auld, 1995; Teketay, 1996; Mbalo and Witkowski, 1997).

Vachellia sieberiana like many other Vachellia spp. in general, is a multipurpose tree providing a wide range of products and services (Pandey and Sharma, 2003). The tree is socially accepted because it provides fuel, fodder, gum, and local medicine to farmers among others (Mugunga and Mugumo, 2013). All parts of the plant are used as medicine. The bark, leaves and gums are used to treat tapeworm, bilharzia, haemorrhage, orchitis, colds, diarrhoea, gonorrhoea, kidney problems, syphilis, ophthalmia, rheumatism and disorders of the circulatory system. It is also used as an astringent. The pods serve as an emollient, and the roots for stomach-ache, acne, tapeworms, urethral problems, oedema and dropsy (Orwa et al., 2010).

Vachellia sieberiana is reported to be one of the most commonly used browse tree species in arid and semi-arid regions of Africa (Ngwa, 2002; Hansen et al., 2008; Ewel et al., 1999) as it has good feeding qualities. It is reported to have low insoluble proanthocyanidins and high soluble phenolics (Woodward and Reed, 1989). Soils under this tree species are often higher in nitrogen, phosphorus and other nutrients (Everett et al., 1986; Belsky et al., 1993; Tiedeman et al., 1993). Acacia litter contributes greatly to soil fertility due to the tree's ability to fix atmospheric nitrogen (Dunham, 1989). In arid and semi-arid areas, the soils beneath the Acacia canopy are usually better developed than those outside the canopy, having higher nitrogen and water contents (Waldon et al., 1989). Nitrogen fixing ability of the tree makes it one of the most suitable species for agricultural fields (Puri et al., 1994).

It is generally observed locally that all Vachellia trees lack representation at the seedling and sapling stage in the field. One possible reason for this may be the absence of bush fires which are prohibited in Rwandan territory for environmental conservation purposes. In an area where wild fire is prohibited, how would the germination of Vachellia species be enhanced? This study aimed to give light on the fate of Vachellia seeds following dispersal. The objective of this study was threefold: (a) to investigate whether seeds retained their viability, b) to identify appropriate seed treatment methods through germination testing c) to assess seedling growth and survival under different soil moisture regimes.

Materials and Methods

Study site

Trials were carried out at Huye Campus of the University of Rwanda, southern Rwanda, located at 02° 36'S and 24°35'E. According to the agro-ecological classification defined by Delepierre (1974), the site is found in the central plateau and hills agro-ecological zone of Rwanda. It has a mean annual rainfall of 1200 mm/year; mean annual temperature of 21oC; altitude ranging from 1500-1600 m altitude; relative humidity of 77% and the soils are derived from granitic rocks and are classified as oxisols or ultisols on the hills (Manzi et al., 2012; Mbonigaba, 2002).

Seed collection and testing

The aims of collection were to verify whether V. sieberiana seedbank was represented also on the soil surface under the tree and to assess the viability of its seeds. For this purpose, seeds were collected on the floor under canopies of mature trees of V. sieberiana trees in Songa pastures (2o 24' S, 29o 46' E; Kinazi sector, Huye district, outhern Province of Rwanda. The site is also in the mid altitude zone (Manzi et al., 2012). Seed collection was done during the short dry season (February) in 2010 when trees were dispersing or had dispersed their seeds. Collected seeds were processed and transported to Huye Campus of the University of Rwanda, where germination tests were subsequently performed.

Seed testing followed International Seed Testing Association (ISTA) standard (Milivojević et al., 2018), in which case, each test comprised a total of 400 seeds in four replications of 100 seeds. Prior to testing, all seeds were sorted out to eliminate those that were infected and/or bearing holes. A sample of seeds was soaked in water for five days to allow for a simple viability test which was done by cutting. Germination testing was done after treating the seed using nine pre-sowing treatment methods of hot wire scarification (HWS), dry heat or seed roasting (R) for different periods of time namely 30 (R30), 40 (R40), 50 (R50), 60 (R60) and 90 seconds (R90) and soaking in cold water (SCW) and in hot water (SHW) for 24 hours. Seed roasting was done by keeping the seeds under constant heat (500oC) in an oven. Treated seeds were germinated in plastic pots filled with river sand and kept moist through daily wetting done in the mornings. Germination counts were made for a period of 16 weeks (4 months) since its commencement.

Inventory of seedlings and saplings

To study the presence or absence of V. *sieberiana* seedlings and saplings in Songa pasture, ten mature seed trees of V. *sieberiana* were selected across the pastures. For each sample tree, circular sample plots of 1 m-diameter were laid along two perpendicular transects running from the tree base to about 15 m away from the crown in north and east directions. This distance was equivalent to the average tree radius meant to represent the area covered by the tree crown. Neighbouring sample plots were laid down along these transects three meters apart. To capture the information on V. *sieberiana* seedling and sapling density, fifty similar plots and equal in size as those described above were laid randomly in Songa pastures in the open areas, representing the area not under tree crowns. In the sample plots, an inventory of all *V. sieberiana* seedlings and saplings was made and recorded.

Nursery studies

Seedlings obtained from the germination testswere used to assess their early survival (S) andgrowth performance in a nursery located at thedepartment of forestry and nature conservation,University of Rwanda Huye campus. Two sets ofseedlings were grown under different conditions:potted seedlings grown in the glass house and bare-rooted seedlings directly pricked out in well prepared plots in the field. Seedlings in the glasshouse were subjected to different watering regimes: daily, once and twice a week at a rate of 60 ml per seedling at each application. Half of the seedlings in the field were completely exposed to the prevailing rain conditions while the other half obtained an additional supplement of 60 ml of water per seedling on days that had no rain.Different growth traits wereassessed. Total height (HTOT) (cm) and rootcollar diameter (RCD) (cm)were measured sixtimes each at a two weeks'interval starting threeweeks after pricking out. The number of leaves (NL), number of leaflets (LL),number of thorns (NTbiomass (W) (g/plant) and root length (RL)were assessed once at the end of the nurseryexperiment. An analysis of variance (ANOVA) was performed and significant meanswere separated using Least Significant Test.

Results and discussion

Seed collection and germination

The level of beetle infection of *V. sieberiana* seed was observed to be 50% since half of them had one or more holes. Larval infestation by bruchid beetles is known to reduce seriously Vachellia seed viability since they destroy many seeds (Andersen et al., 2015; Ward et al., 2010; Or and Ward 2003), which was estimated up to 0-82% (Walters and Milton, 2003; Ernst et al., 1989).

Tests were made on sample seeds after removing those that were infected. *V. sieberiana* seed displayed a high level of dormancy. Seed treatments that involved hot wire scarification (HWS) and soaking in water (SCW & SHW) significantly enhanced germination than the rest of the treatments ($p<0.05$). Germination of untreated and roasted seed did not differ significantly and all rates were below ≤8%. After four months of observation, the germination rate ranged from 5.5% in the control through to 72% for SCW, 86% for SHW to 90% for HWS treatments (Figure 1).

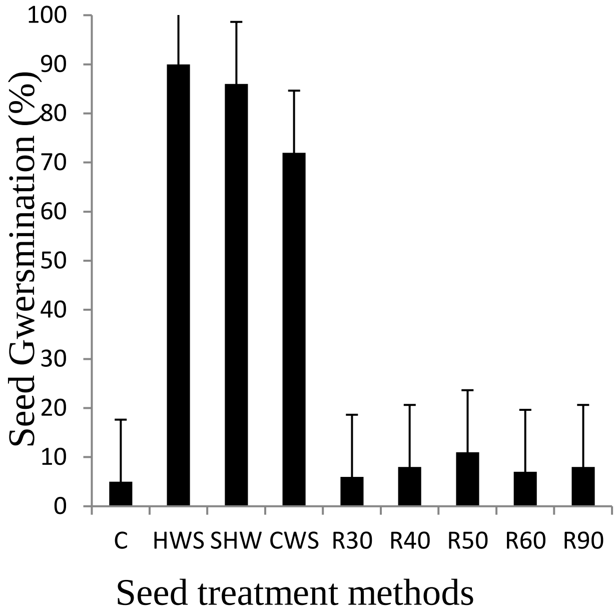


Fig.1 Germination rates of *Vachellia sieberiana* seeds obtained byusing nine seed treatment methodsduring sixteen weeks, and seedscollectedundernatural conditions on thetree crownflowin Songa pastures, southern Rwanda.Seed treatments included no treatment (C), hotwire scarification (HWS), soaking in hot water (SHW), soaking in cold water (SCW) androasting for 30 (R30), 40 (R40), 50 (R50), 60 (R60) and 60 (R90) seconds

Although seed germination was highly improved by some treatments namely HWS and soaking in water, the germination was very sporadic and it took up to 16 weeks to attain the highest value recorded. Water treatments had a more uniform germination compared to HWS treatment but this also took a long time than would be desirable. This is demonstrated by cumulative germination rates where most seed germinated during the first eight weeks, a time when only a few HWS treated seed had germinated. At eight weeks for example, germination rates were about 41, 54 and 75% for HWS, SCW and SHW respectively.

Contradicting results have been reported in different places. Soaking seeds in water in Vachelliaspp. in Zambia led to results comparable to the findings in this study (Chidumayo, 2008) while dry heat treatment gave germination rates of only 20% for *V. sieberiana* and 53% for *V. gerrardii* in Uganda (Mucunguzi and Oryema-Origa, 1996).

The highlevel of dormancy observed was most likely due toimpermeable seed coat as commonly observed inmost legumes (Gashaw and Michelsen, 2002). Seeddormancy is however an ecological strategycommon to most of the legume species native toarid or semi-arid areas (Munyanziza and Musanga,1996). Unfortunately, prolonged seed dormancybecomes a handicap to mass propagation inreforestation programs (Koutouan-Kontchoi et al.,2020).

Germination rate for water or heat treated seeds in Vachellias is generally low but this may be attributed to the high levels of seed infection. Many Vachelia species are reported to show substantial reduction in seed germination under bruchid beetles infestation (Rohner and Ward, 1999). This can be illustrated by the present study which used clean, undamaged seed. The observation is further supported by findings by Warrag and Eltigan (2005) who compared the germination rates of clean and unclean seed and found that, at each soaking period, the clean seeds had higher germination percentages followed by the unclean seeds. Wang and Eltigan (2005) reported 80-100% germination for clean seed and observed that 100% germination could be achieved if clean seeds were kept under high shade and soaking in water for longer periods. This gave comparable results with soaking Vachellia seed in concentrated sulphuric acid to break seed dormancy, where rates of up to 85 % have been attained (Mohamed & Abdel-Majed, 1996).

The sporadic nature of germination in the Vachellias such as observed in this species still needs to be addressed. Our findings are consistent with previous studies (Chin and Robert, 1980; Chidumayo, 2008). There is still a room for improvement of the germination percentage and speed through the identification of more efficient seed treatment methods.

Inventory of seedlings and saplings

Irrespective the presence of sound and viable seeds under each of the investigated Vachellia tree, only one few weeks old seedling was observed in the study area of over 310 ha. The absence of seedlings and saplings may be partly explained by the dormancy observed in Vachellia seeds. Antagonistic environmental conditions including seed predation or damage by pests, climatic conditions and browsing or trampling by animals might play a role as well. The poor natural regeneration in tropical trees has been observed in Africa (Chidumayo, 2008). This increases the vulnerability of Vachellia species since they are generally not fully integrated in tree planting programs.

Effects of watering regime on seedling performance

Dry matter production

Shoot dry matter production was positively correlated to watering intensity, that is the higher the intensity, the higher the shoot biomass ($p<0.05$) (Figure 2). Daily watering was observed to increase shoot and root weight in other species, example, Solanum macrocarpon (Ogunrotimi and Kayode, 2018). Usually plants growing in areas with ample water supply invest in shoot production so as to exploit light resources. As for root production, increasing moisture stress tends to produce fine roots which contribute very little to underground biomass and which are yet more efficient in nutrient and water uptake (Munyanziza, 2001). Usually, water stress reduces total biomass in most tree species and the rate of biomass reduction increases with the level of water stress (Zavala and Rayetta, 2001; Villagra and Cavagnaro, 2006; Pizarro and

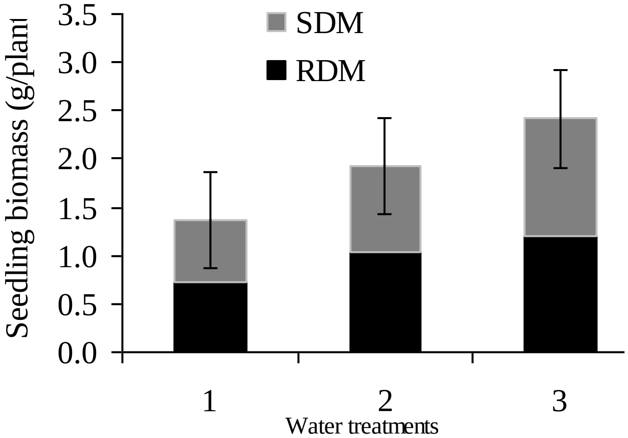
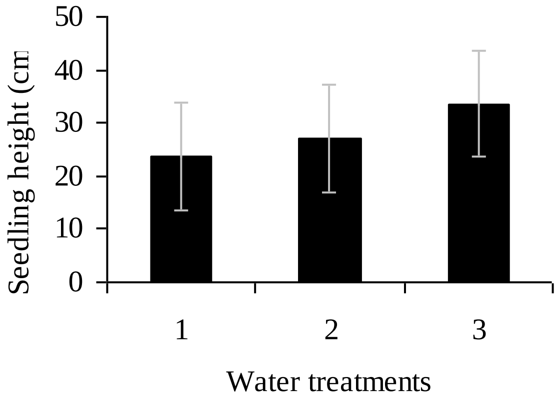


Fig.2 Total dry matter allocation in V. *sieberiana* seedlings as a function and shoot dry matter (SDM)respectively

Height

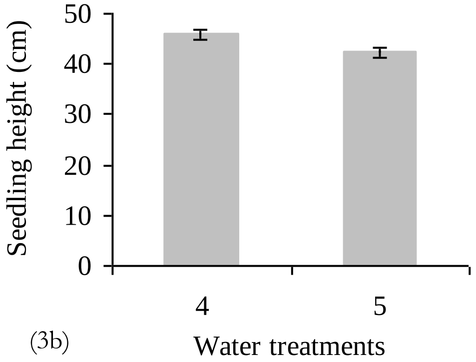
Watering intensity significantly ($p<0.05$) increased seedling growth and the difference was higher in potted seedlings than in bare-rooted seedlings ($p<0.05$). Height growth ranged from 24 cm in seedlings that received water once a week to 34 cm for those that receiving water daily, an increase of about 30% (Figure 3a). There was no significant difference in height growth in bare-rooted seedlings between those receiving additional water and those relying merely on rainfall (Figure 3b). One of the functions of the taproot in arid or semi arid plants is to grow deep to tap water from the ground (Munyanziza, 2001).

This was possible in the field but not in the potted seedlings whose root growth is restricted by containers and/or management through root pruning. Thus root management is important for seedling growth and survival. Another explanation is that bare rooted seedlings benefited from rain water as the experiment was run in theopen space during the rain season. Water deficit is often a key factor limiting plant growth,resource allocation patterns and survival, especiallyin semiarid areas subject to episodes of prolongeddrought (Boyer, 1982). There was sufficientmoisture from rainfall and additional water did notaffect growth significantly (Fig. 3).



(3a) Potted Seedlings

Fig 3. *V. sieberiana* seedling height growth as affected by watering intensity. Potted seedlings (a) received three different treatments: 1. watering once/week, 2. watering twice/week and 3. normal watering of twice daily. Bare-rooted seedlings

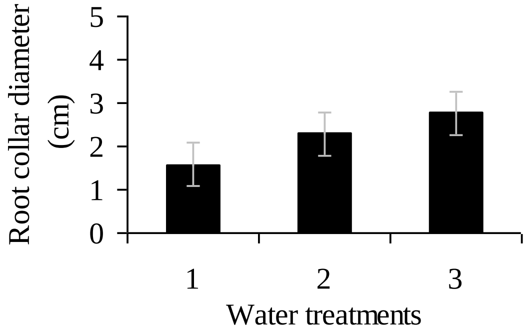


(3b)

(3b- bare rooted seedlings) received two treatments of 4. rain water and 5. rainwater plus extra watering with 60ml of water on rainless days.

Root collar diameter

The trend in root collar diameter, number of leaves and thorn production followed the same line as seedling height. Root collar diameter increased significantly with increasing watering regime ($p<0.05$) (Figure 4a). Bare-rooted seedlings grew 42% bigger than potted seedlings (Figure 4b). This underlies once again the importance of free root development in seedling growth.



(4a) Potted seedlings(above); (4b) bare -rooted seedlings(below)

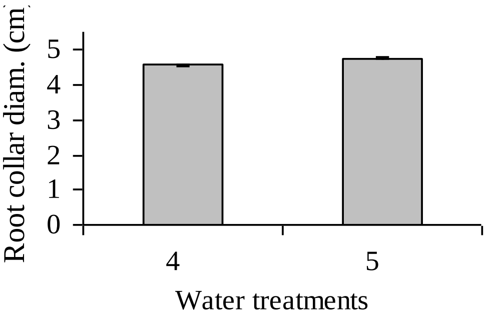
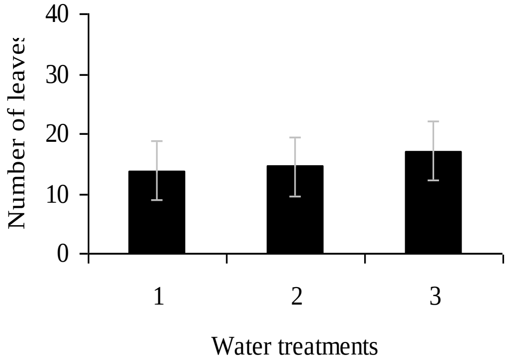


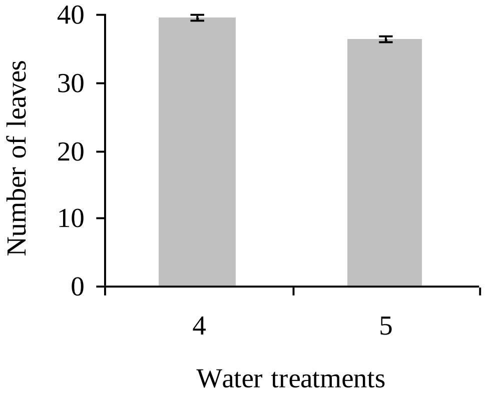
Fig. 4. Root collar diameter in V. s*ieberiana* as affected by watering regime. Water treatments as in Figure 3

Number of leaves and leaflets

The number of leaves and leaflets was positively and significantly enhanced by the intensity of watering ($p< 0.001$) (Figure 5). Reduced production of leaves as a response to reduced soil moisture content is another ecological strategy to decrease water losses through transpiration (Munyanziza, 2001).



(5a) Potted seedlings

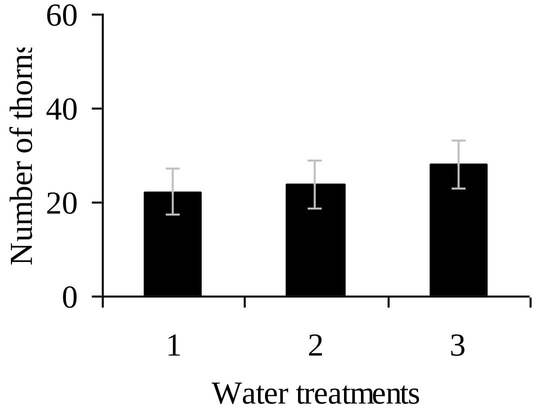


(5b) bare-rooted seedlings

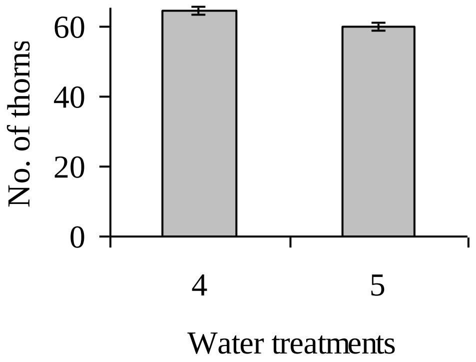
Fig. 5 Number of leaves and leaflets as affected by watering intensity. Water treatments as explained in Fig.3

Number of thorns

Watering intensity enhanced positively and significantly the production of thorns ($p<0.05$) (Figure 6). As it was generally observed, every leaf was guarded by a pair of thorns. This shows the importance of leaves for a plant as photosynthesizing organs.



(6a) Potted seedlings



(6b) Bare-rooted seedlings

Fig. 6 Thorn production in 16 weeks old *V. sieberiana* as affected by watering regime. Water treatments as described earlier.

Root length

While moisture stress negatively affected shoot growth, it significantly increased root length ($p<0.05$) (Figure 7). Root length or specific root length (measured on secondary roots) is a function of the amount of fine roots and is used to describe the ability of species to search for soil volume per unit mass invested, usually promoted in water-stressed environment (Nicotra et al., 2002; Cornelissen et al., 2003). This is in commensurate with reports in other places where plants in water stressed environments allocate more biomass to the roots compared to the shoots (Markesteijn and Poorter, 2009). This is an ecological strategy which is used to take up more water (Munyanziza, 2001). Root extension rate and specific taproot length are reported to be higher in tree species originating from semi-arid than those from humid environments (Tomlinson et al., 2012). The promotion of fine roots in moisture deficient soils contrasts with the loss of, or reduced rate of leaf production in shoots when water is limited. Both fine roots and leaves are involved in uptake and synthesis of growth nutrients needed by plants.

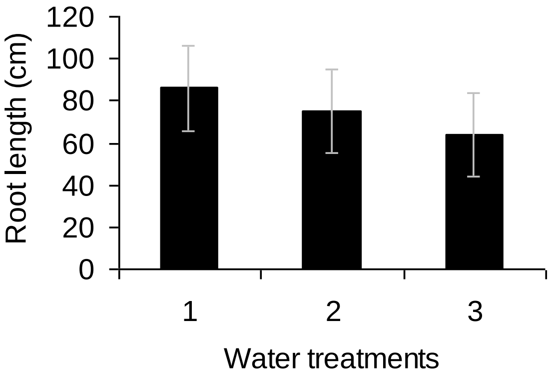


Fig. 7 Root length (RL) in *V. sieberiana* seedlings as affected by moisture stress. Treatments1, 2 and 3 as described earlier

Arid areas are also characterised by high rainfall variation resulting from erratic and poorly distributed rainfall patterns during the growing season than is the case in wetter, humid environments (Ananthakrishnan and Soman, 1989; Nicholls and Wong, 1990; Ward, 2009). As water percolates in deeper layers rendering top soils drier in arid environments, young seedlings fight to reach soil water to avoid death due to water stress by increasing root length among other strategies (Bond 2008). Two traits are observed to be important for accessing deeper water: fast root extension rates (mm day-1) and efficient depth penetration per unit cost in root biomass, achieved by producing longer and thinner taproots or specific taproot length (Tomlinson et al., 2012). Root extension rates and specific taproot length might both be greater among species abundant in drier environments than species abundant in wetter environments (Tomlinson, 2012). This may explain the high growth rate observed in *V. sieberiana* root length.

Conclusion

Vachellia sieberiana has a big regeneration potential owing to its production of large amounts of seeds. Surprisingly however, the species was representedin the field by only mature trees with a general lackof seedlings or saplings, probably due to beetle infection and seed dormancy. Seed treatment by scarification improved germination rates up to 90% against 6% recorded in untreated seeds. Hot water treatment equally increased seed germination rate (observed to be 86%) and the germination speed with the latter was higher than in hot wire treatment. Since hot wire scarification is very difficult and only applicable in small seedlots for research purposes, soaking V. sieberiana seed in hot water was identified as the best treatment method in this study and it is recommended for the species. However, seed germination was still sporadic and took a long time to reach 90%. Further seed testing is still required to identify seed pre-treatment methods which may reduce sporadic germination observed in this species. The failure of roasting treatment to improve germination rejects the hypothesis that "no bush fires" leads to the poor or no regeneration of the species by seed. *V. sieberiana* seedlings showed a reasonably fast nursery growth rate reaching a plantable size (≥30 cm in height) within 2-3 months. This high survival rate under field conditions may be attributed to its taproot-dominated root system and its quick response to moisture stress by the production of large population of fine roots which increased manifold its root length and presumably soil water and nutrient uptake. It is recommended to adopt V. *sieberiana* among species used in the reclamation of waste lands to exploit its potential in agroforestry systems

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