https://doi.org/10.26525/jtfs2021.33.3.349

SEED BALL TECHNIQUE FOR ENHANCING THE ESTABLISHMENT OF SUBABUL (*LEUCAENA LEUCOCEPHALA*) UNDER VARIED HABITATS

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Submitted August 2020; accepted December 2020

Deforestation and forest degradation are the biggest threats to our forests. Afforestation is one way to sustain life and combat global warming. Unfortunately, high cost and hazard factors in handling of tree saplings and initial care after planting affect the success rate of afforestation projects. Seed ball technique is effective for propagating plants from seeds because the seeds are protected from external stress and predation. The present experiment investigated the applicability of this technique in afforestation programmes at three different locations for the establishment of subabul (*Leucaena leucocephala*), a tree fodder, which is a major source of grazing for forest animals. Seed balls was prepared using red soil and vermicompost at a ratio of 2:1 and mixed with 230–250 mL of water per kg of medium. Seed balls protected the seeds from biotic and abiotic stresses and the vermicompost provided nutrients and growth hormones which greatly improved the establishment and survival rate of subabul. Seed ball technique is recommended for forest regeneration and re-greening the earth surface.

Keywords: Fodder tree, road sides, forest area, river bank, improving green cover

INTRODUCTION

The global temperature is rising due to the increased emissions of greenhouse gases including carbon dioxide, causing extreme climatic irregularities. One of the major reasons for the rise in greenhouse gases is oxygen–carbon dioxide imbalance due to deforestation. According to FAOSTAT (2019) forest area has reduced by 0.5% over the last 10 years and this has contributed to the 11% increase in atmospheric carbon dioxide. If this condition continues, it will lead to an oxygen-deficient earth and the only way to overcome this problem is afforestation.

Deforestation also leads to lower or non-availability of forage and fodders for grazing animals, which also affects growth and production of agricultural crop. In the Amazon forests, low-cost direct sowing using seed balls of various tree, shrub and grass species were successfully carried out in regenerating destroyed regions (Jakovac et al. 2016, Balkrishna et al. 2020). Most regeneration techniques (e.g. planting of saplings and trees, cuttings) are not cost effective and this has been a major barrier for up scaling this activity (De Groot et al. 2013, Jayawardhane & Gunaratne 2020).

To increase green coverage in forest areas through natural regeneration, the seeds of various species are sown directly in the forest areas which are usually difficult to get to. However, the seeds are usually eaten by birds and animals or carried away by wind and water, resulting in poor seedling establishment in forest areas. Aerial seeding also results in poor survival besides being very expensive. Another reason for the high mortality rate of directly sown seeds is because roots from germinated seeds fail to penetrate the upper surface of the soil. Seed predation is listed as the most common reason for failure for direct seed planting in Illinois (Farlee 2013).

Seed ball is a cost-effective technique useful for revegetating large areas of arid regions (Atkinson 2003). Clay seed balls can be utilised for planting trees, shrubs, wildflowers and vegetables. To ensure success of large-scale forest restoration, the methods used must be ecologically friendly and cost effective and should involve local stakeholders (Shoo et al. 2016, Holl 2017).

Seed balls provide burying condition without the need to dig a hole for each seed. Seed balls can be easily made from any seed weighing less than 1 g. Fukuoka (2009) reported that seed balls prepared from a combination of loamy soil, compost manure, water and rice seeds perform better after being dry sown in semi-arid and arid areas. Seed balls provide microsite protection from predation and adverse environmental conditions and the organic content in the medium provides basic sources to the germinating seeds during seed germination. Seed balls are scattered throughout degraded lands and will sprout when it rains and conditions are just right for germination (Fukuoka 1985).

Subabul (*Leucaena leucocephala*) is one of the fast growing hardy evergreen species with vigorous coppice shoot, and strong and deep taproot, which can withstand land slopes and control soil erosion. Subabul has prolific seed production and the leaves have high nutritional value, making good fodder. The regeneration capacity of this species is high and it is also drought resistant (Parthiban et al. 2018). Therefore, subabul can be a good candidate for increasing the green cover of forested areas. Apart from forests, this species can be used to green road sides and river banks that have lost their trees due to urbanisation. Considering these facts, this study has been formulated to evaluate subabul seed balls under different environmental conditions.

MATERIALS AND METHODS

Experimental area

Seed balls were prepared at the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore. Field experiments were conducted in October 2018 at three different locations in Krishnagiri district, Tamilnadu, viz., road side, forest area and the bank of Thenpannai River, each with two water regimes (irrigated and non-irrigated). These three locations were selected because trees in these areas are being cut down to make way for other landuses. The average weather parameters of Krishnagiri district at that time are presented in Table 1.

Preparation of seed ball

Subabul seeds, red soil and vermicompost were obtained from the university. The red soil was sieved to get fine powder for easy handling and to produce seed balls with smooth surfaces. Two parts of sieved red soil were mixed with one part of vermicompost and was thoroughly mixed to get fine powder. After mixing, 230–250 mL of water was added to 1 kg of seed ball medium to get optimum moisture in order to obtain seed balls with proper size and shape. Each seed ball costs 0.95 rupee (USD0.013) to make.

Since subabul seeds exhibit hard seededness up to 87%, the seeds were scarified with concentrated sulphuric acid at 100 mL kg^{-1} of seeds for 10 min. After acid scarification, the seeds were thoroughly washed with water and shade dried for 18 hours. Direct sun drying was avoided to reduce crack lines and breakage during handling, packaging and transportation of seed balls. Two or three subabul seeds were placed inside 10–15 g of well-mixed seed ball medium to make a ball. The balls were dried in the shade for 24 to 36 hours depending on the atmospheric conditions (Figure 1).

A total of 1000 each of seeds (control) and seeds balls were manually broadcasted in the selected locations (road side, forest area and

Table 1 Weather parameters during the period of experiment at Krishnagiri in October 2018

Weather parameter		Value
Rainfall (mm)		257.8
Relative humidity (%)		70
Temperature (°C)	Max	30
	Min	21
	Avg	26
Wind speed (km $hour^{-1}$)	Max	14.1
	Avg	13.1
Sun hours (hours)		200.5
Snow fall (cm)		0

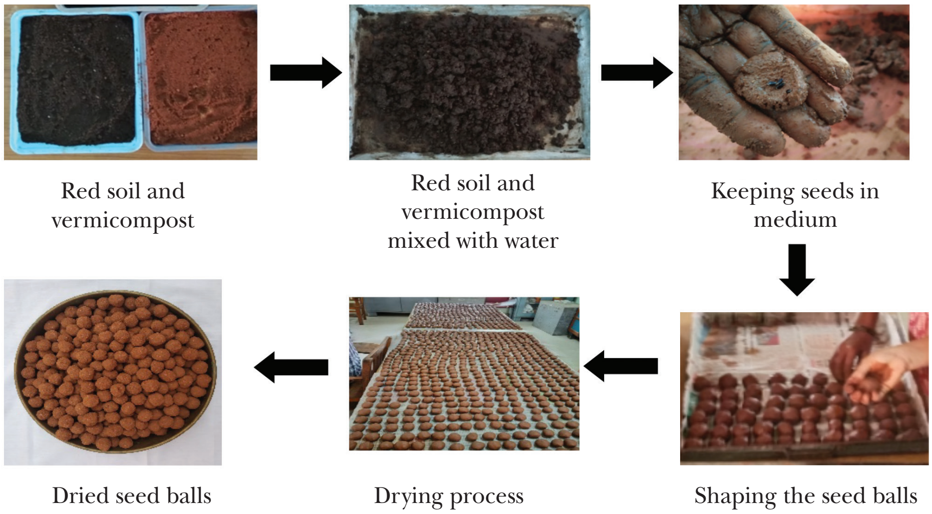

Figure 1 Prepartion of subabul seed balls

river bank). Separate areas, each 110 m^2, were marked for control and seed balls. The sites were maintained under two water regimes (with or without irrigation). Irrigation was done manually using rose can once every other day. Data on germination (%), root length (cm), shoot length (cm), vigour index and dry matter accumulation (mg 10 $seedlings^{-1}$) were recorded for the whole of October and analysed.

Statistical analysis

The analysis of variance and Duncan's multiple range test was carried out to compare germination of control and seed balls as well as germination in the different water regimes and locations. The mean difference was significant at the p values < 0.05. Statistical analysis was performed using the SPSS 16.0 software.

RESULTS AND DISCUSSION

There were positive influences of seed balls on seedling quality parameters in all environments and conditions (Tables 2–4).

Effect of seed balls on seedling quality at the road side

Maximum germination (93%) was observed in seed balls with irrigation (Table 2). Increased germination up to 13.4 and 97.8% was observed in irrigated seed balls over irrigated and non-irrigated controls respectively. Germination of seed ball without irrigation (56%) was higher than control (47%), showing that seed ball had protected the seeds from animals and helped the seeds to germinate.

Table 2 Effects of subabul seed balls on physiological seed quality parameters with different water regimes at the road side

Irrigation	Treatment	Germination (%)	Root length (cm)	Shoot length (cm)	Dry matter production (mg 10 $seedlings^{-1}$)	Vigour index
With irrigation	Control	82 (64.89) ± 0.89^{bab}	9.6 ± 0.06^{bab}	7.5 ± 0.06^{bab}	0.604 ± 0.02^{bab}	1402 ± 3.07^{bab}
	Seed balls	93 (74.65) ± 0.87^{aaa}	11.2 ± 0.02^{aaa}	7.7 ± 0.05^{aaa}	0.791 ± 0.03^{aaa}	1758 ± 6.26^{aaa}
Without irrigation	Control	47 (43.28) ± 0.63^{bbd}	6.8 ± 0.03^{bbd}	5.4 ± 0.01^{bbd}	0.498 ± 0.01^{bbc}	573 ± 7.21^{bbd}
	Seed balls	56 (48.44) ± 0.90^{abc}	7.1 ± 0.05^{abc}	5.9 ± 0.09^{abc}	0.501 ± 0.02^{abc}	728 ± 7.68^{abc}

Figures in parentheses indicate arcsine values; data presented are means of four replicates with standard errors, within each treatment, different letters in each column indicate significant differences by Duncan's multiple range test at p < 0.05

Root length of the road side seeds ranged from 6.8 to 11.2 cm and shoot length, from 5.4 to 7.7 cm. With irrigation, root and shoot length increased 16.7 and 2.7% respectively over control (Table 2). The least dry matter production was observed in control seeds without irrigation (0.498 mg 10 seedlings^{-1}). Irrigated seeds balls showed an increase of 58% over control without irrigation. Similar trend was observed in vigour index with maximum value of 1758 in seed balls with irrigation (Table 2).

Effect of seed balls on seedling quality at the forest area

Under forest area, germination percent ranged from 40 to 89% (Table 3). Maximum germination was observed in seed balls grown in irrigated condition.

The highest of root and shoot lengths were observed in seed balls under irrigated condition (12.1 and 9.5 cm respectively). Root and shoot lengths were least in control under non-irrigated condition (9.2 and 6.3 cm respectively). Between the highest and lowest values of dry matter production and vigour index, there were differences of 43.1 and 210.0% in seed balls over control under irrigated condition (Table 3). Lowest dry matter accumulation and vigour index were recorded in non-irrigated control (0.614 and 620 mg 10 seedlings^{-1} respectively). When compared with non-irrigated condition, all parameters performed well in irrigated condition.

Effect of seed balls on seedling quality at the river bank

Germination was lowest in control seeds under non-irrigated condition (43%) while maximum germination of 90% was recorded in seed balls under irrigated condition (Table 4). Greatest root length was obtained from seed balls under irrigated condition (11.8 cm) and lowest was in control under non-irrigated condition (9.9 cm). An increased percentage of 15.6% was obtained in root length over control under irrigated condition. Similar trends were observed in shoot length with values ranging from 6.8–9.0 cm.

Dry matter production ranged from 0.641–0.842 mg 10 seedlings^{-1} and vigour index, 718–1872 (Table 4). Dry matter and vigour index in irrigated seed balls increased by 31.3 and 160.7% respectively compared with non-irrigated control. As with the other two locations, highest values were obtained when using seed balls with irrigation.

Direct sowing of tree seeds for afforestation is not practical due to several biotic and abiotic constraints. Most species record low establishment rates in the natural environment because seeds get over dried and this leads to death of seeds. Other factors are predation by natural enemies and poor root penetration of seedlings (Ceccon et al. 2016, Satya et al. 2018). Seed ball technique has been proven to improve seedling quality parameters. In this study, seed balls improved growth of seedlings under irrigated and non-irrigated (rain-fed) conditions.

Table 3 Effects of subabul seed balls on physiological seed quality parameters with different water regimes at the forest region

Irrigation	Treatment	Germination (%)	Root length (cm)	Shoot length (cm)	Dry matter production (mg 10 seedlings^{-1})	Vigour index
With irrigation	Control	83 (65.64) ± 0.85^{bab}	10.5 ± 0.13^{bab}	8.6 ± 0.06^{bab}	0.659 ± 0.01^{bac}	1585 ± 13.48^{bab}
	Seed balls	89 (70.63) ± 0.33^{aaa}	12.1 ± 0.15^{aaa}	9.5 ± 0.02^{aaa}	0.879 ± 0.02^{aaa}	1922 ± 10.95^{aaa}
Without irrigation	Control	40 (39.23) ± 0.12^{bbd}	9.2 ± 0.14^{bbc}	6.3 ± 0.07^{bbc}	0.614 ± 0.01^{bbd}	620 ± 9.91^{bbd}
	Seed balls	52 (46.14) ± 0.14^{abc}	11.8 ± 0.11^{aba}	8.5 ± 0.12^{abb}	0.792 ± 0.01^{abb}	1055 ± 6.36^{abc}

Figures in parentheses indicate arcsine values; data presented are means of four replicates with standard errors, within each treatment, different letters at each column indicate significant differences by Duncan's multiple range test at $p < 0.05$

Table 4 Effects of subabul seed balls on physiological seed quality parameters with different water regimes at the river bank

Irrigation	Treatment	Germination (%)	Root length (cm)	Shoot length (cm)	Dry matter production (mg 10 seedlings^{-1})	Vigour index
With irrigation	Control	85 (67.21) ± 1.10^{ba}	10.2 ± 0.11^{babc}	8.1 ± 0.13^{bab}	0.634 ± 0.01^{bac}	1555 ± 7.68^{bab}
	Seed balls	90 (71.56) ± 1.22^{aa}	11.8 ± 0.18^{aaa}	9.0 ± 0.16^{aaa}	0.842 ± 0.01^{aaa}	1872 ± 4.16^{aaa}
Without irrigation	Control	43 (40.97) ± 0.72^{bb}	9.9 ± 0.10^{bbb}	6.8 ± 0.09^{bbc}	0.641 ± 0.01^{bbc}	718 ± 3.19^{bbd}
	Seed balls	47 (43.28) ± 0.58^{ab}	10.5 ± 0.16^{abc}	8.9 ± 0.14^{aba}	0.783 ± 0.01^{abb}	912 ± 3.72^{abc}

Figures in parentheses indicate arcsine values; data presented are means of four replicates with standard errors, within each treatment, different letters in each column indicate significant differences by Duncan's multiple range test at $p < 0.05$

Rising temperature and reduced rainfall cause abiotic stress including prolonged drought and heat waves resulting in rapid changes in environmental conditions. During hot weather, direct seeding will cause loss of seed viability through over drying of seed moisture. Increased temperature causes osmotic stress in plant cells and seeds directly sown on the surface of soil (Suzuki et al. 2014, Kollist et al. 2018). Soil compactness due to adverse environmental condition such as drought causes seed death, and cold and chilling injuries to seed (Alameda et al. 2012, Rivera et al. 2019). Under natural conditions, direct seeding will lead to loss by biotic factors, e.g. infections by pathogens and predation by pests such as birds, ants and rodents (Atkinson & Urwin 2012, Farlee 2013, Suzuki et al. 2014). All these factors cause low establishment and survival rate of the seeds used in afforestation programmes. Seed ball protects seeds from these natural biotic and abiotic stresses by providing a protective layer and also maintains seed viability and seed quality without any losses.

Pre- and post-germination quality parameters of the seed depends on activation of enzymes and availability of essential nutrients and hormones. Essential nutrients and hormones during seed development act as trigger mechanisms for seed germination. Seed germination requires temperature, water, light, enzymes and essential nutrients. During medium standardisation, seeds from seed balls germinate earlier compared with control seeds due to the effect of solid matrix priming during drying of seed balls which was 24 to 36 hours under shade condition. During this period seeds slowly absorb moisture from the seed ball medium and the process of germination is initiated. Improved germination was also due to the humic acid, nutrients, organic substance and other plant growth hormones present in vermicompost and red soil.

Water plays a major role in seed germination, which is a primary trigger through imbibition and activation of hormones and enzymes. Therefore, greater seedling emergence was observed when seed balls were irrigated due to the easy and fast imbibition of water by seeds which initiates earlier activation of hydrolytic enzymes. Red earth and vermicompost improve seedling germination, vigour and length (Chiranjeevi et al. 2018, Sondarva et al. 2018). The vermicompost present in seed balls also act as water absorbing medium because decayed organic contents hold water and presence of hormonal substance like humic contents leads to timely germination (Arancon et al. 2008, Lazcano et al. 2010).

Increased root length was registered in seedlings from seed balls due to the availability of nutrients that facilitated proper root development. The seed balls also had phosphorus from the compost. Minimum root length was noticed in control seeds due to problems in root penetration after germination because the seeds were sown on the surface of soil, whereas in seed balls, sowing process is completed during seed ball preparation itself. In all the three different environmental conditions, increased germination and seedling length, higher amount of dry mass accumulation and seedling vigour were obtained from seed balls due to the presence of organic matters and other essential micronutrients. Fischer et al. (2010) reported that root formation between irrigated and rain-fed seeds and seed balls was significantly different. Nutrient uptake was greater due to higher number of secondary and lengthy roots that can absorb nutrients from the soil and subsequently supply them to growing seedlings (Ali et al. 2017). Combined application of red earth and vermicompost increased seed germination, root and shoot length and biomass accumulation.

Between the three different locations, the highest rate of establishment and survival in terms of maximum germination and seedling vigour was obtained in forest area followed by river bank and road side. This was due to favourable climatic condition as rainfall and other weather parameters were positive determinants for better germination and seedling development. The microclimate in the forest influenced the rate and growth of plant establishment. There were differences in parameters between irrigated and non-irrigated conditions in all the three locations. The highest germination and seedling quality were registered under irrigated condition because of the high amount of water available from manual irrigation and from rainfall while the lowest germination was under non-irrigated condition in all the three locations. Even without irrigation, better germination and improved seedling vigour were observed from seed balls, where the organic contents in the seed ball acted as water absorbing medium.

CONCLUSIONS

From this study, the seed ball technique proved to be low cost and can be used to recover vegetation in deforested areas. This technique can overcome biotic and abiotic stresses which hinder seed germination and viability. It can be effectively utilised for better seedling establishment, vigour and survival even in resource-limited conditions. Under non-irrigated condition, seed ball performed better then control. The nutrients in the seed ball medium provided additional nutrients and growth promoting factors for enhancing the establishment and survival rate of the seedings.

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