Influence of Sowing Depth and Shade on Emergence and Seedling Growth of *Centrosema pubescens*

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Abstract: Depth of sowing and shade levels were two ecological factors affecting early establishment of a plant. The effects of sowing depths of seeds and shade on seedling emergence, morphological traits and seedling biomass of *Centrosema pubescens* were examined. The seeds were sown at the depth of 2, 4, 6 and 8 cm and shaded at levels of 0 (full sunlight) and 50%. Analysis of variance showed a significant effect ($P<0.05$) of sowing depth on seedling performances. Sowing at the depth of 2 cm produced the highest percentage of seedling emergence, the shortest days to emergence, the biggest seedling diameter and the greatest seedling biomass. Shade significantly ($P<0.05$) increased seedling height and shoot-root dry weight ratio. The findings indicate that for improved growth and dry matter yield, *Centrosema pubescens* seeds should be sown at a depth of 2 cm.

Keywords: Sowing depth, shade, *Centrosema pubescens*, seedling performances.

1. Introduction

Centrosema pubescens (centro) is a perennial twining trailing legume that can climb nearby grasses and fences. It is native to sub-humid and humid regions of New World tropics and now has been naturalized in tropical Asia and Africa, from sea level to an altitude of 1,660 m. In Indonesia, it is grown as plantation ground cover, in mixture with grasses for grazed pastures, as cut and carry forage and as green manure. Centro has a high potential adaptation to diverse habitats such as the dry and high altitude of tropics and subtropics, poorly drained and/or seasonally flooded conditions and acids, low fertility soils (Schultze-Kraft *et al*., 1990).

As animal feed, centro contains a high nutritive value. It contains 22.1% crude protein, 5.1% ash, NDF 53.2%, ADF 40.1% and in vitro dry matter digestibility 52% (Valarini and Possenti, 2006). Due to its high nutritive value, centro is often used to improve nutritive value of low quality of tropical grasses, both by introducing to grass pasture or mixed with grasses in their diets.

Centro is very suitable to be introduced to grasses stand because it is compatible with many grasses (Cakraborty and Mandal, 1999; Baba *et al*., 2011) and in some grass-legume mixtures; it is relatively resistant to heavy grazing compared to other legumes (Olanite *et al*., 2004). In introducing legumes into grassland stand, good establishment is essential. To ensure such a good crop, high levels emergence and seedling growth are important parameter to allow a good competition against weeds and a rapid growth against competing grasses.

Rapidity and emergence percentage have been shown to be influenced by sowing depth. Sowing depth at an optimum depth is an important aspect to obtain higher emergence and good quality of seedlings. Sowing at shallow depth generally stimulates more seed germination and seed emergence than seeds sown on the soil surface, because the former provides a moist environment around them and prevents seeds and seedlings from drying out, as well as preventing damaged by insects.

Deeper sowing usually delays and reduces emergence and vigor of seedlings, frequently leads to poor establishment, despite more deeply sown seeds having a greater chance of accessing soil moisture (Buakum *et al*., 2013). Excessive sowing depth prevents seed from emerging above the soil surface and thus prevents their survival. Therefore, determining optimum planting depth for individual species is critical for establishing productive stands in the field.

Emergence and seedling growth in the field are also related to light level. Light is generally known as one of the ecological factor exerting the greatest effect on germination, seedling emergence and development (Baiyeri, 2006). The ability to grow rapidly when shaded is an important adaptation mechanism when species deprived of their full complement of sunlight (Bjorkman *et al*., 2000). Both in pure and mixture with grasses, competition for light from weeds and grasses will inhibit the establishment of legume in pastures. It has been reported that legume inter-seeding into grassland is most successful when competition from grasses is reduced through herbicide application or by grazing swards to low height before seeding legumes (Teutsch and Fike, 2003).

Seed germination and emergence is species specific. Seeds of some species germinate and emerge equally in light and dark (Norsworthy and Oliveira, 2005), some require light to stimulate seedling growth (Li *et al*., 2012; Kalmbacher, 2013), and others are favored by low light intensity (Gardener *et al*., 2001; Jellicorse *et al*., 2009).

There is limited documentation on the effect of depth of sowing and shade on emergence, growth and biomass production of centro. Such information is useful to provide agricultural extension delivery to farmers for increased forage productivity. The present study was designed to determine the effects of sowing depths of seeds and shade on emergence and seedling growth of centro.

2. Materials and Methods

2.1. *Experimental Site and Materials*

A pot experiment was conducted in June 2013 in grassland experimental field of Faculty of Animal Science, Hasanuddin University Makassar, Indonesia (5°14' S, 119°42' E) with 7 m elevation above sea levels. The mean annual rainfall was 1,465 mm per year which falls mainly from December until March which accounts for more than 60% of the total annual rainfall.

Seeds of *Centrosema pubescens* were obtained from plant growing naturally in the campus of Hasanuddin University South Sulawesi, Indonesia, one month before experiment started. After air drying, seeds were stored under temperature of 4^{o} C. The floatability method was used to assess seed viability. The seeds were placed in a 1 litre container that was later filled with water; floating seeds were assumed to be unviable, while sunken seeds to be viable. The floating seeds were discarded from the experiment.

2.2. *Experimental Procedure*

This study was arranged in split plot completely randomized design, with two light levels (shade 50% and full sunlight) as the main plot and sowing depth (2, 4, 6 and 8 cm) as subplot with three replications, making 24 pots in total. The pots (14 cm in height, bottom diameter of 12 cm and top diameter of 16 cm) were first filled with soil to a certain depth at which 40 seeds each were placed. The pots then filled with additional soil to obtain the desired planting depth. The soil was clay loam texture of ultisol soil that had been passed through a 5 mm sieve. Artificial shade – 50% of full sunlight was provided by black plastic screen fixed in polyvinyl frames with dimension of 215 x 80 x 38 cm and placed under field conditions.

The pots were watered with tap water to field capacity. Once a day, pots were checked for seedling emergence. Seedlings were thinned to five plants per pot after full cotyledon extension stage was attained. Cumulative emergence over the entire duration of experiment was measured.

Experiment was terminated at 16 days after sowing when cotyledon began to yellow. At harvest, all seedlings were measured for percentage of emergence, rapidity of emergence, seedling height, seedling diameter, number of leaves, shoot and root biomasses, seedling biomass and shoot-root ratio. Emergence of seedling was noted as the first appearance of any portion of seedling above soil surface. Stem height of seedling was measured from soil surface to the tip of the uppermost stem, using a ruler. The diameter of stem seedlings was measured with micrometer screw gauge at 1 cm above soil surface.

At harvest, soil was washed from below ground hypocotyl and roots in running water. Roots from below ground hypocotyl were removed and mixed with basal roots. Each shoot and roots of seedlings were oven dried at 65^{o}C for 72 hours to determine their biomass dry weight. Shoot seedling biomass was measured as dry weight of seedlings minus roots.

2.3. *Data Analysis*

SPSS version 16 statistical software were used to analyze the effects of light levels, sowing depth sand their interaction on emergence percentage, rapidity of emergence, seedling height, seedling diameter, number of leaves, shoot and root biomasses, seedling dry weight and ratio of shoot to roots. To meet assumption of ANOVA, data for percentage of emergence were arc-sin-transformed prior to analysis. Least significant difference (LSD) was used to compare treatment means.

3. Results and Discussion

3.1. Seedling Emergence

A two way ANOVA showed that emergence percentage was significantly affected by sowing depth, but not by shade and interaction between sowing depth and shade (Table 1). Seedling emergence occurred at all sowing depth, however seedling emergence was the highest at 2 cm sowing depth and then decreased with increasing sowing depth (Table 2). Increasing sowing depth from 2 cm to 8 cm reduced seedling emergence by 34.33%. Poor emergence at deep sowing is consistent with that of Agbo (2012) and Aikins *et al*. (2006) that deep sowing can significantly affect crop emergence and yield and this may be attributed to relatively more favorable oxygen, light, temperature and soil moisture conservation capacity at shallower depth.

It seems a greater fraction of seed reserves was exhausted by the time of seedling emergence because seedling that emerged from deeper sowing depth showed a smaller size.

The highest seedling emergence at 2 cm of sowing depth indicates that seedlings of centro had the highest potential for successful establishment at that depth. Emergence is an important event that affects the success of crop. Rapid, uniform and complete emergence of vigorous seedling, leads to higher yield potential by shortening the time from sowing to complete ground cover, allows the establishment of optimum canopy structure to minimize plant competition, maximize yield and provide plants with time and spatial advantages to compete with weeds (Soltani *et al*., 2001).

The days to emerge were significantly longer with increasing sowing depth (Table 2), which is also in line with Ren *et al*. (2002) and Juan *et al*. (2011). For all sowing depth, the fastest seedling emergence occurred from 2 cm sowing depth and delayed with increasing sowing depth. Seeds sown at 8 cm depth took significantly longer time to emerge than those at 6, 4 and 2 cm sowing depth.

Delaying of emergence at deeper sowing might be due to seedlings requiring more energy resources to penetrate the thicker soil layer after germination from greater depth and thus required more time before

Table 1. Influence of sowing depths, shade levels and their interaction on emergence, morphological characteristics and seedling biomass of *Centrosema pubescens*.

Parameter	Sowing depth	Shade	Sowing depth x Shade
Emergence percentage	**	NS	NS
Days to emergence	**	NS	NS
Seedling height (cm)	**	*	NS
Stem diameter (cm)	NS	*	NS
Number of leaves (/plant)	NS	NS	NS
Shoot biomass (g/plant)	*	*	NS
Roots biomass (g/plant)	*	NS	NS
Seedling biomass	*	*	NS
Shoot-root ratio	NS	*	NS

*P <0.05 ** P <0.01 NS indicates not significant finally emerging from the soil surface and probably more susceptible to establishment failure (Burmeier *et al*., 2010). This implies that "shallow emerger" like centro has a competitive advantage over seedlings originating from greater depths because they can start assimilating before the others have even reached soil surface, which may increase their establishment chances.

There was no significant difference between unshaded and shaded plants on emergence percentage (Table 1). However, data from Table 2 shows that emergence of shaded plants was higher than that of unshaded plants, which consistent with previous works (Baiyeri, 2006; Gardener *et al*., 2001). Becker *et al*. (1988) noted that seeds under full sunlight might have experienced both heat and moisture stress in the upper layer of the soil. However, the days to emergence in this study was not influenced by shade levels.

3.2. *Morphological Traits*

The morphological traits, except number of leaves were significantly influenced by sowing depth and/or shade (Table 1). Seedling height was the greatest at 2 cm of sowing depth and decreased with increasing sowing depth (Table 2). Similar effect of sowing depth on seedling height and seedling diameter has been reported by Seeiso and Materechera (2011) and Wu *et al*. (2011). Lower seedling height at deeper seeding might be attributed to longer seedling emergence as influenced by difficulty of seedlings to push their shoots through the thick soil layer.

There was a significant effect of shade on seedling height and seedling diameter (Table 1) in which seedling height was greater under shade than under full light (Table 2). This is consistent with other studies which show that in a light-limited environment, photosynthetic allocation patterns favor shoot elongation and hence, increase light harvesting capabilities (Wang *et al*., 1994). However, the greater seedling height under shade in this study occurred at the expense of seedling diameter that was significantly lower under shade. This study corroborates the findings reported by Akhter *et al*. (2009) and Mayoli *et al*. (2009). Apical dominance tends to increase when plants are subjected to shade, due to a decrease in the production of photosynthetic and the high levels of auxin at the stem apex bud (Woodward and Bartel, 2005).

There was no significant effect of sowing and shade on number of leaves. However, number of leaves was higher under full light than under shade conditions. Wadud *et al*. (2002) and Cookson and Granier (2006) observed similar trend of number of leaves under shade. This may be due to lower production of photosynthetic under low light levels.

3.3. *Seedling Biomass*

There was a significant effect of sowing depth and shade on shoot biomass, root biomass and seedling biomass (Table 1). Shoot, root and seedling biomasses were the highest at 2 cm of sowing depth and grown in full light conditions and decreased with increasing of sowing depth and lowering light intensity (Table 2) and this was similar to that reported by Paul *et al*. (2012) and Wu *et al*. (2011). Decreased seedling biomass when sowing depth increased may be due to

Table 2. Emergence, morphological traits and seedling growth of *Centrosema pubescens* as influenced by sowing depths and shade

Treatments	Percentage of emergence	Days to emergence	Seedling height (cm)	Seedling diameter (cm)	Number of leaves per plant	Shoot biomass (g/plant)	Root biomass (g/plant)	Seedling biomass (g/plant)	Shoot-root dry weight ratio
Sowing depth									
2 cm	57.465^{d}	2.671^{a}	4.330^{c}	0.773^{a}	4.800^{a}	0.143^{c}	0.023^{b}	0.166^{d}	6.217^{a}
4 cm	52.025^{c}	3.002^{ab}	3.750^{c}	0.903^{a}	4.930^{a}	0.124^{bc}	0.019^{a}	0.143^{c}	6.526^{a}
6 cm	42.080^{b}	5.173^{b}	3.000^{b}	0.893^{a}	4.670^{a}	0.116^{b}	0.017^{a}	0.127^{b}	6.834^{a}
8 cm	37.920^{a}	6.005^{b}	2.200^{a}	0.863^{a}	4.930^{a}	0.099^{a}	0.015^{a}	0.114^{a}	6.566^{a}
Shade									
0%	44.58^{a}	4.167^{a}	2.010^{a}	1.037^{b}	0.154^{a}	0.126^{b}	0.025^{b}	0.154^{b}	4.500^{a}
50%	52.28^{a}	3.250^{a}	4.480^{b}	0.806^{a}	0.125^{a}	0.113^{a}	0.012^{a}	0.125^{a}	9.416^{b}

Means of each parameter followed by different letters in each column are significantly different at $P < 0.05$ level.

slower time to emergence and hence delaying time to start photosynthesis.

Both shoot biomass and root biomass were depressed by shade, leading to a decreased of seedling biomass. However, root biomass was more depressed under shade. Shade reduced shoot biomass by 11.50% while roots biomass by 64%, which implies that under shade conditions plants allocated more of their dry matter to shoots than to roots growth and development.

Shoot biomass and root biomass were both influenced by sowing depth and shade. The allometric relationship between shoot growth and root growth as expressed by shoot-root dry weight ratio in different seeding depth and light levels are shown in Table 2. The results were not significantly different between sowing depths but shoot-root ratios in shaded plants were significantly higher than that of unshaded plants.

The relatively high shoot and root ratio in shade-grown plants may be an environmentally induced adaptation which permits higher rates photosynthesis under low light intensity. The high shoot-root ratios indicated that seedlings under shade conditions located more of their dry matter to shoot than to root and this may decrease resistance of plant to draught conditions, because the higher shoot-root ratio reduced draught tolerance in crop plants (Eshanullah *et al*., 1999).

4. Conclusion

It can be concluded that sowing seed at 2 cm significantly improved in the percentage of emergence, time to seedling emergence, seedling diameter and seedling biomass weight. The shade produced improvement in seedling height, shoot biomass and shoot-root ratio. Considering the soil and weather conditions of the experiment, centro seeds should be sown at a depth of 2 cm to obtain the best growth and dry matter yield.

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