Research Article

Study on Biochemical Differences in Sett Material of BN Grass and Its Influence on Sprouting and Establishment

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Abstract

A Field experiment was carried out at research farm, AC and RI, Coimbatore during *rabi* season 2015-2016 to assess the biochemical differences in sett material from different portions of bajra napier grass stem. The experiment was laid out in Randomized Block design with three replications. The treatments comprised of seven different sett material used for planting *viz*., single budded sett with top portion, middle portion, bottom portion and two budded sett with top portion, middle portion, bottom portion and rooted slips. The results of this study showed positive impact on biochemical analysis of planting material before planting. Results revealed that top portion recorded higher reducing (25.89 mg g^{-1}, 12.54 mg g^{-1}) and non reducing sugar (6.71 mg g^{-1}, 31.67 mg g^{-1}), total sugar (32.6 mg g^{-1}, 42.1 mg g^{-1}), starch (25.6 mg g^{-1}, 19.9 mg g^{-1}) and lower total phenolics (2.61 mg g^{-1}, 3.13 mg g^{-1}) and IAAO activity (66.9, 73.8 µg of unoxidised auxin g^{-1} hr^{-1}) in both node and internode region of stem portion than bottom portion. Also significantly higher number of sprouting (95.3%) and establishment (100%) from the sprouting percentage were observed with rooted slips.

Keywords: Bajra Napier, establishment percentage, germination percentage, IAAO activity, non reducing sugar, reducing sugar, planting material, rooted slips, sprouting %, starch, total phenolics

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Introduction

The forage resources in India are mainly derived from crop residues, cultivated forages and grazing from pastures and grasslands. Bajra Napier (BN) hybrid is one of the important and high yield perennial fodder crop among different grass fodders cultivated. It is vegetatively propagated through stem cuttings (setts) for its production in fields. Sprouting is major potent factor contributing toward final fodder-yield. It has special significance in vegetatively propagated crops like BN hybrid grass where poor sprouting creates gaps, which adversely affect tonnage. Good sprouting and 100% establishment of the sprouted buds lays the foundation of the subsequent ratoon crop [1]. Thus, uniform plant stand is prerequisite to ensure optimal yield across the environment. Therefore, sprouting and establishment studies will have to be continued with a view to take maximum possible advantages of the fodder bud planted. This requires that our research should be based on both biochemical and field observations of the problem.

For sprouting and establishment age of buds and reserved food materials plays an important role in any crop. Under field conditions, sprouting starts from 3 days after planting (DAP) and completed within 20 DAP. Establishment observed up to 30 DAP. The sprouting is a biological process which depends on the internal factors like starch, total sugar (Reducing and Non reducing sugars), total phenolics content and Indole-3-acetic acid activity (IAAO). Initial amount of starch, total sugars are mobilized in buds to provide building blocks and energy for its sprouting [2]. It is important to emphasize that some sugars (glucose and sucrose) are important signaling molecules that affect plant sprouting, growth and development [3]. Total phenolics are physiologically active secondary metabolites produced by higher plants which had the property to increase the indole-3-acetic acid (IAA) destruction [4] and stimulated the decarboxylation of IAA [5]. The IAA oxidase activity determines the auxin levels and thereby apical dominance. IAA oxidase activity was low in the region of high auxin content and high in the region of low auxin content [6].

Apart from many other factors which influence on sprouting and subsequent growth of vegetatively propagated crops stem cutting used as seed is the prime factor [7]. Higher sprouting percentage in cuttings taken from the upper portion, and the time required for sprouting was also much lesser [8] when compared to cuttings taken from the middle and bottom portions [9]. Till date, studies performed are very meager under different planting material (i.e., difference in no of buds and different portion) and biochemical aspect to understand the reason for reduced sprouting% and establishment in BN grass. Hence, the present study was undertaken with an objective to find out the suitable portion of planting material to get higher fodder yield.

Materials and Methods

A Field experiment was conducted during *rabi* season of 2015-2016 at Research Farm, Agricultural College and Research Institute, Coimbatore, Tamil Nadu. General view of experimental field is shown in (**Figure 1**). The experimental site is geographically located in the Western Agro Climatic Zone of Tamil Nadu at 11°N latitude, 77 °E longitude with an altitude of 426.7 m above mean sea level. The soil of the experimental site was sandy clay loam in texture having alkaline pH (8.48) and low organic carbon (0.25%), With regard nutrient status, the soil was low in available nitrogen (242.4 kg ha^{-1}), medium in phosphorus (20.5 kg ha^{-1}) and high in potassium (675.0 kg ha^{-1}), respectively. Rice variety CO (BN) 5 with the duration of 135 days was used as test crop. The experiment was laid out in randomized block design with three replications. The treatments comprised of seven different planting material *viz*., single budded sett - top portion, single budded sett - middle portion, single budded sett - bottom portion, two budded sett - top portion, two budded sett - middle portion, two budded sett - bottom portion and rooted slips are shown in the (**Figure 2**). Sett piece ranging between 12 – 15 cm and 30 – 40 cm length stem in either side of the node was cut with sharp knife for single and two budded sett. The sett pieces for single and two budded sett top middle and bottom portions were taken from the whole stem which is equally divided as top, middle and bottom portions. Rooted slips are taken from the existing field tussocks where stem cuttings were taken. Slips are already rooted and hence planted along with roots.

Figure 1 General view of the experimental field during planting and crop period

In order to evaluate the effect of different planting material on biochemical parameters, sprouting, and establishment percentage the data were statistically analyzed using "Analysis of variance test". The critical difference at 5% level of significance was calculated to find out the significance of different treatments over each other [10]. Gross plot size 5.4 m x 5.0 m and net plot size 3.0 m x 3.0 m. Recommended dose of N, P and K (150: 50: 40 kg/ha) were applied in the form of urea, single super phosphate and muriate of potash, respectively. Half dose of N and full dose of P and K were applied basally to all the treatments and the remaining 50% N was top dressed at 30 DAP. After each cut nitrogen @ 75 kg/ha was applied. Hand weeding was done as and when required. The first cutting was made on 78 days after planting and the subsequent cutting was carried out at an interval of 45 days. The first cutting was made on 78 days after planting and the subsequent cutting was carried out at an interval of 45 days. The total number of BN grass setts that sprouted were counted up to 20 DAP (Days after planting) for each plot. The percentage of setts that established up to 30 DAP was counted for each plot. Sprouting and establishment percentage at 20 DAP and 30 DAP was worked out for all the plots and expressed as percentage. The biochemical parameters were estimated by taking samples in both node and internode region of the setts and methods followed were, total sugars by anthrone method [11], reducing sugars by Nelson-Somogyi method [12], starch content by anthrone method [11], total phenolics content by method of [13], non reducing sugar content was arrived by subtracting reducing sugar from total sugar and all the parameters are expressed in mg g^{-1} of fresh weight. IAAO activity of planting material was assayed before planting and two days after sprouting using the method suggested by [14]. The enzyme activity was expressed as µg of unoxidised auxin $g^{-1} h^{-1}$.

Figure 2 Different planting material used for the study

Reducing sugar content

The reducing sugar content was arrived by employing the formula suggested by [12] and expressed in mg g^{-1} of fresh weight.

Non reducing sugar content

The amount of non-reducing sugars of planting material was arrived by subtracting reducing sugar from total sugar and expressed in mg g^{-1} of fresh weight.

Total sugar content

The total sugar content was total soluble sugars of planting material was estimated by anthrone method suggested by [11] and expressed in mg g^{-1} of fresh weight.

Starch content

The total starch content of planting material was estimated by anthrone method suggested by [11] and expressed as mg g^{-1} of fresh weight.

Total phenolics content

Total phenolics content of planting material was estimated by the method suggested by [13] and expressed as mg g^{-1} of fresh weight.

Indole-3-acetic acid oxidase (IAAO) activity

The IAAO activity of planting material was assayed before planting and two days after sprouting using the method suggested by suggested by [14] using Garden-Weber reagent. The enzyme activity was expressed as µg of unoxidisedauxing^{-1}hr^{-1}.

Results and Discussion

Influence of Biochemical Parameters on Germination and Establishment

Reducing and non reducing sugar

Non reducing and reducing sugar content of the plant gradually decreased from top to bottom portion of the stem (**Tables 1** and **2**). Internode samples from top portions registered significantly higher non reducing sugars of 31.67 mg g^{-1} than other portion and node region. The higher content of non reducing sugar in internode region may be due to internodes are the location where sugar in the form of non-reducing sugars is translocated. Similar results were reported by [15]. Since node region is the storing part, the non reducing sugars may be converted to reducing sugars by enzyme may be the reason for higher reducing sugar content in the internode. Incase of reducing sugars, top portion of node region recorded higher values of 25.89 mg g^{-1} than internode region of top portion. Similar trend noticed in other portions has also been reported by [16] and [17].

Table 1 Non reducing sugar content (mg g^{-1}) in different stem portions of bajranapier hybrid grass

	Plant sample	Non reducing sugar (mg g^{-1})	
		Nodes	**Internodes**
1	Top portion	6.71	31.67
2	Middle portion	4.67	26.58
3	Bottom portion	2.53	19.12
	SEd	0.32	0.83
	CD(p=0.05)	0.91	2.29

Total sugar and starch

The total sugar content of the plant also gradually decreased from top portion to bottom portion of the stem, whereas it is *vice-versa* in starch content both in node and internode samples of all portions (**Tables 3** and **4**). Node and internode samples from top portion registered peak values of 32.6 and 42.1 mg g^{-1} but starch content with lower values of 25.6 and 19.9 mg g^{-1}. The more accumulation of total sugars in top portion might be due to increased activity of enzymes *viz*., Invertase, alpha amylase, sucrose synthase in immature tissue during sprouting has also been confirmed by [18] and [15]. Also the higher starch content in bottom portion than other portion due to accumulation of fixed carbon as starch in amyloplasts, the storage sinks. These results are correlated with by [19] and by [20] in sugarcane.

Table 2 Reducing sugar content (mg g^{-1}) in different stem portions of bajranapier hybrid grass

	Plant sample	Reducing sugar (mg g^{-1})	
		Nodes	**Internodes**
1	Top portion	25.89	12.54
2	Middle portion	20.63	10.98
3	Bottom portion	17.52	6.53
	SEd	0.84	0.43
	CD(p=0.05)	2.35	1.19

Table 3 Total sugar content (mg g^{-1}) in different stem portions of bajranapier hybrid grass

	Plant sample	Total sugar (mg g^{-1})	
		Nodes	**Internodes**
1	Top portion	32.6	42.1
2	Middle portion	25.3	37.2
3	Bottom portion	20.1	25.4
	SEd	1.5	1.7
	CD(p=0.05)	4.1	4.8

Table 4 Starch content (mg g^{-1}) in different stem portions of bajranapier hybrid grass

	Plant sample	Starch (mg g^{-1})	
		Nodes	**Internodes**
1	Top portion	25.6	19.9
2	Middle portion	48.5	31.2
3	Bottom portion	57.5	48.2
	SEd	2.5	1.9
	CD(p=0.05)	6.8	3.3

Total phenolics and IAAO activity

The phenolics content of the plant also increased from top to bottom portion of the stem (**Tables 5** and **6**). The nodes and internodes from top portion registered lower total phenolics content of 2.61 and 3.13 mg g^{-1} respectively than other portions. Before planting, top portion recorded significantly lower IAAO activity with higher unoxidised auxin of 66.9 and 73.8 µg of unoxidised auxin g^{-1} hr^{-1} in node and internode regions. Whereas the bottom portion recorded higher IAAO activity measured with lower unoxidised auxin in node and internode regions with values of 20.6 and 25.4 µg of unoxidised auxin g^{-1} hr^{-1}. Unoxidized auxin decreased at bottom portions due to higher IAA oxidase activity. [4] also reported that IAA oxidase activity was low in the region of high auxin content and high in the region of low auxin content. Similar findings were reported by [6] that phenolics had the property to increase the IAA destruction. This similar trend was also indicated by [21] and [22].

Table 5 Total phenolics content (mg g^{-1}) in different stem portions of bajranapier hybrid grass

	Plant sample	Total phenolics (mg g^{-1})	
		Nodes	**Internodes**
1	Top portion	2.61	3.13
2	Middle portion	3.14	3.82
3	Bottom portion	4.05	4.67
	SEd	0.17	0.20
	CD(p=0.05)	0.46	0.57

Table 6 Indole-3-acetic acid oxidase (IAAO) activity (µg of unoxidised auxin $g^{-1}hr^{-1}$) in different stem portions of bajranapier hybrid grass

	Plant sample	IAAO (µg of unoxidised auxin g^{-1} hr^{-1})		
		Before planting		Two days after sprouting
		Nodes	Internodes	sprouted bud
1	Top portion	66.9	73.8	77.0
2	Middle portion	32.1	36.1	44.9
3	Bottom portion	20.6	25.4	29.1
	SEd	1.5	2.2	2.1
	CD(p=0.05)	3.9	5.2	4.5

Sprouting and establishment percentage

The rooted slips (T_7) recorded 95.3 per cent of sprouting and establishment which is significantly superior than other treatments (**Table 7**). This might be due to the already established root system which starts its function to absorb moisture and nutrients from the soil immediately after planting. Similar, observations were also recorded by [23] in BN hybrid grass. Among sett portions, two budded sett top portion registered significantly higher sprouting of 83.9%. However, two budded sett bottom portion recorded higher establishment of 80.7%. The superiority in sprouting of two budded setts over single budded sett is due to higher number of buds which in turn enhanced the chance of sprouting. These results are in line with [24] and [25]. The superiority of middle portion of the stem in establishment over top portion could be attributed to its ability to withstand harsh environment in the field [26]. The lower sprouting and establishment percentage of 39.6 per cent was recorded in single budded sett bottom portion (T_3). This might be governed by factors like lower total sugar, higher starch, total phenolics content and higher activity of IAAO which negatively affect the sprouting percentage and recorded 39.6% sprouting in single budded sett bottom portion [27].

Table 7 Effect of different planting materials on sprouting and establishment percentage of bajra napier hybrid grass

	Treatment	Sprouting (%)	Establishment (%)
T_1	Single budded sett - top portion	62.6 (77.6)	54.3 (65.6)
T_2	Single budded sett- middle portion	60.7 (75.5)	60.7 (75.5)
T_3	Single budded sett - bottom portion	38.9 (39.6)	38.9 (39.6)
T_4	Two budded sett - top portion	67.2 (83.9)	62.8 (78.6)
T_5	Two budded sett - middle portion	64.5 (80.7)	64.5 (80.7)
T_6	Two budded sett - bottom portion	43.5 (47.4)	43.5 (47.4)
T_7	Rooted slips	78.3 (95.3)	78.3 (95.3)
	SEd	4.3	3.1
	CD (p=0.05)	9.4	6.7

Figures in parenthesis are orginal values – arcsine transformation was carried out

Conclusion

Even though rooted slips registered higher sprouting and establishment percentage. Due to high seed material cost, more labour requirement, difficulty in uprooting rooted slips from tussocks are practical constraints associated with collection of rooted slips as planting material. As an alternative among the setts, two budded sett of middle portion registered highest establishment percentage 80.7 with higher yield will be a viable alternative planting material.

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