DR-1714
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RESEARCH ARTICLE Asian Journal of Dairy and Food Research

Bud Chip Method: A Potential *In vitro* Technology for Bajra-napier Hybrid (*Pennisetum glaucum* L. X *P. purpureum* Schum.) for Sett Multiplication

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10.18805/ajdfr.DR-1714

ABSTRACT

Background: Forages of high quality are the most valuable commodity of any livestock activity and they serve as the basis for most of the rations in a forage-based diet. The lack of good quality seedlings/seeds, notably improved varieties, is the primary reason for the slow adoption of improved forage production technologies. Traditional establishment of bajra napier forage incurs more labour costs, harvesting sett material, transporting, preparing two budded setts and carrying to the planting area. Nearly three to four tons of green leaves stem materials are wasted for planting one hectare of area. In this situation, an alternate method is required to produce high-quality seedlings in a short period. This research objective compared single node bud chip seedling in different protray cell cavities and pot mixtures.

Methods: The green shade net field study was carried out between 2019 and 2020, the effect of different pro-tray sizes (50, 60 and 98 cell cavities) and potting mixture (red earth, cocopeat, farmyard manure, vermicompost and root growth promoters like phosphobacteria bio-fertilizer and indole acetic acid (IAA) was taken in different combination for the establishment of Bajra Napier hybrid variety Co (BN)-5 seedling by using single node bud chip technology. The observation was taken up to 30 days in related to seedling growth and development. The observed parameter was statistically analysed in SPSS software.

Result: Among the three different protray cavities and five different pot mixtures, 60 cavity protray with red earth + cocopeat + vermicompost (2:1:1) results revealed significant differences ($p>0.05$) in seedling growth parameters among the different protray and growing media. The maximum seedling growth parameters like germination, numbers of the leaf, plant height, leaf length, width and root length (94.4 per cent, 8.17, 39.12 cm, 38.14 cm, 2.5 cm and 30.8 cm) were observed at 30 days aged seedlings followed by red earth + cocopeat + farmyard manure (2:1:1 ratio) and treated with IAA substance in 60 cavity protray.

Key words: IAA, Protray, Pot mixture, Single node bud chip bajra napier, Vermicompost.

INTRODUCTION

Livestock is a sub-sector of agriculture that contributes significantly to nutritional security, particularly for small and marginal farmers. The livestock population is increasing due to the continuous effort and implementation of various schemes and projects by the central and state governments. We have approximately 12.9 per cent of the world's cattle, 54.6 per cent of the world's buffaloes, 6.2 per cent of the world's goats and 14.4 per cent of the world's sheep population (Basic Statistics of Animal Husbandry and Dairying, 2019). However, our animals' productivity is significantly lower than the global average due to various factors, the most serious of which is a lack of feed and fodder. The country currently has a green fodder deficit of 63.5 percent and a dry crop residue deficit of 23.5 per cent Dunna *et al*. (2014). The main explanation is the scarcity of high-quality seedlings/seeds and the slow implementation of improved forage production technologies. Several fodder varieties have produced, but seeds are not available at the farmer's door, limiting the fodder area's expansion.

In the traditional process, Bajra Napier was planted with two-node stem cuttings called setts, which incurs additional labour costs for harvesting the stalk, transportation to the field, preparation of two budded setts and transportation of the prepared setts to the planting area. Besides, approximately three to four tonnes of green leaves and stem materials are lost when planting one hectare of land. Under field conditions, it had a low germination percentage after planting. As a result, alternative seed/ seedling development methods must be implemented to increase the fodder cultivating field.

The protray nursery is an emerging technique for quality seedling development in which seedlings are grown under a shade net and have better germination, appear healthy,

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How to cite this article: Senthilkumar, D., Paramasivam, A., Ramachandran, M. (2021). Bud Chip Method: A Potential In-vitro Technology for Bajra-napier Hybrid (*Pennisetum glaucum* L. X *P. purpureum* Schum.) for Sett Multiplication. Asian Journal of Dairy and Food Research. DOI: 10.18805/ajdfr.DR-1714.

Submitted: 11-05-2021 **Accepted:** 08-09-2021 **Online:** 06-10-2021

are protected from pests and diseases and establish a well-developed root system within 25-30 days of planting (Bryant, 1995.). The role of the protray nursery unit is to minimize seedling mortality by growing elite healthy seedlings and supplying them to the farming community (Anonymous, 2003).

Dunna *et al*. (2014) discovered that closely planted setts in an upright position with one node within the soil at a distance of 5 cm x 5 cm row-to-row and plant-to-plant. The shoot buds began to turn into leafy shoots within ten days and roots emerged after 15 days. In 4 to 5 weeks, the setts were ready for transplanting with proper root and shoot growth.

The protray method of nursery seedling production is an efficient commercial production method. It is highly profitable and effective for large-scale production and commercialization (Yadav and Bajpay, 2019).

The survival of the plant is typically dependent on favourable environmental factors and one of the most critical factors needed for the development of high-quality seedlings is growing media. Sarker *et al*. (2014) conducted an in-vitro wheat seedling bioassay analysis with two superior PSB isolates (PSB1 and PSB8) and different sources of P. They discovered that both isolates significantly improved seedling growth (shoot and root length, shoot and root dry weight) and nutrient contents (NPK per cent) in plant tissue when compared to controls (no PSB).

Cocopeat contributes to the proper texture of the growing media and prevents compaction, resulting in better root and shoot growth. It may be due to the cocopeat medium's higher water holding capacity, aeration and usable organic matter (Nair and Bharathi, 2015)

Padhiyar *et al*. (2017) discovered that chrysanthemum growth cocopeat with vermicompost and bio compost provided the best growth due to the media's favourable physiochemical properties, *i.e*., improved water relationships, nutrient retention, allows free air movement and maintains moisture.

Akat *et al*. (2017) investigate the effects of various rooting media and naphthalene acetic acid on the rooting abilities and shoot growth of Nerium oleander cuttings. The results showed that peat was the best rooting medium and 500 mg of NAA per lit of water increased the rooting ratio by 60% and the addition of NAA increased shoot fresh and dry weight.

However, standardization of the protray and potting mixture is needed for large-scale production of single node bud chip Bajra Napier seedlings. The current study was carried out to determine the combined effect of different protray cavities and pot media proportion on the development of Bajra Napier seedlings in protray.

MATERIALS AND METHODS

Location and climatic condition

During 2019 - 2020, the experiment was conducted at the Livestock Farm Complex, Veterinary College and Research Institute, Orathanadu (Latitude: 10° 37' 0.12" N Longitude: 79° 16' 0.12" E and 2 m above sea level). Orathanadu block. During the summer months of April and May, the maximum temperature is 38.50°C. The winter months are January and February, with a minimum temperature of 29.72°C. The annual rainfall average is 987 mm.

Treatments and experiment design

The experiment was carried out in a strip plot design with three replications to investigate three protray cavities' combined effect (M_1-50 cell cavities, M_2 - 60 cell cavities). M_3 - 98 cell cavities, as well as five separate pot mixtures Subplot: Pot mixture media, specifically (S_1 - Red earth + Cocopeat + Farmyard manure (2:1:1 ratio), S_2 - Red earth + Cocopeat + Vermicompost (2:1:1 ratio), S_3 - Red earth + Cocopeat + Farmyard manure (2:1:1 ratio) and treated with Phosphobacteria bio-fertilizer, S_4 - Red earth + Coco pit + Farmyard manure (2:1:1 ratio), S_5 - Red earth alone (control). The experiment was replicated twice, first from 20th December to 18th January and second from 1st of February to 2nd of March 2020.

Planting materials

Among the various forage crops studied, the Bajra-Napier hybrid (*Pennisetum glaucum* L. X *P. purpureum* Schum.) variety BN (Co-5) was chosen because it is easy to root, produces more adventitious root and is a high promoting fodder crop due to its high yield potential as compared to other fodder crops. The healthy stalk material was chosen from a four-month-old crop. By using a bud chipper, single nodal stem cuttings with bud lengths of at least 5 cm and thicknesses of 1.5 - 2.0 cm were prepared (Fig 1). The prepared single bud was planted in the protrays upward direction.

Protray

Trays usually measure 54.0 cm in length and 27.0 cm in width. Three different protray cavity numbers were used, namely 50, 60 and 90. The cavity depth in each protray is 4.0, 8 and 3 cm, respectively and the spacing between the single bud after planting is 2.5, 3.5 and 1.5 cm (Fig 2). It is reusable and made of polypropylene. Each cavity in the trays has pre-punched holes for proper drainage of excess water.

Pot mixtures

The potting mixture's selection and proportion depending on the type of crop, availability of mixtures, duration of seedling, water requirement and local situation. A well-sieved

Fig 1: Preparation of single node bud.

(2 mm size) red sandy-loam soil, well-decomposed cocopeat, farmyard manure and vermicompost were used as the constituents of a potting mixture as a rooting pot mixture.

Single node bud chip cuttings treating with root growth-promoting substances

The prepared single-node stem cuttings of 5 - 6 cm length was immersed in a solution of NAA 0.5 per cent concentration (5 mg in one litre of distilled water) for 10 minutes and *Phosphobacteria* biofertilizer (10 gm/lit of water) root growth-promoting substances as per the treatment (S_3 and S_4). After soaking, single node buds were collected and used for germination in a protray.

Planting of single node chip bud in protray

The different potting mixtures were filled in half of each cell in the three forms of protrays based on the treatments. In each cell of the protray, a single budded node was placed and trays were filled with potting mixtures and gently pressed according to the treatments (Fig 3). The planted protrays are kept in a green shade net environment. They were watering the individual trays twice a day, in the morning and evening, for 30 days.

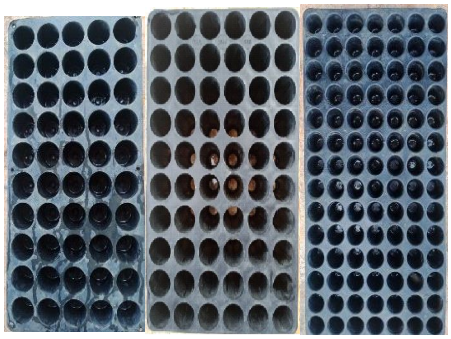

Fig 2: Protray of 50, 60 and 98 cavities.

Fig 3: Planting of single node bud in portray.

Fig 4: Overall view of germination in different protray cavities and pot mixtures.

Observation

Observations on growth parameters, *viz.*, germination percentage, plant height, number of leaf/seedlings, leaf length, leaf width and root length were recorded at 15 DAP and 30 DAP.

Germination percentage

The germination percent of seedlings with different growth media were calculated using the following formula:

$$\text{Germination (\%)} = \frac{\text{Number of buds germinated}}{\text{Total number of buds sown}} \times 100$$

Growth parameters

The growth parameters like plant height, leaf length, leaf width and root length were measured on a scale (cm) unit. The number of leaves per plant was counted in numbers.

Statistical analysis

All data were analyzed using SPSS 27 (SPSS Inc., Chicago, IL, USA) of IBM (IBM Corporation, North Castle, NY, USA). To assess the importance of key and interaction results, use the Statistics Base module. The data was plotted and the points' differences were compared using standard error. Under the general linear model, the multivariant test was used to compare all possible treatments and worked out the interaction effect by using correlation at $P<0.05$.

RESULTS AND DISCUSSION

Growing media influences seed germination, succeeding emergence and growth of seedlings in a nursery because it is a reservoir of moisture and plant nutrients. From the results of Table 1, the maximum germination (83.53%) was observed in protray having 60 cavity cells and treatment received a potting mixture of red earth + cocopeat + vermicompost (2:1:1) of 83.37 per cent. The average value of germination percentage component-wise significantly differs with the protray and potting mixture. In Table 2, the highest germination was found to be 94.11 per cent, with the combination of protray having 60 cavities and a potting mixture of red earth + cocopeat + vermicompost (M_2S_2) (Fig 4). The lowest germination was observed at 39 per cent, with 98 cavities protray having red earth alone. The media increases the water contact, nutrient retention, allows free air movement, retains moisture and nutrients for plant growth, most likely due to optimum bulk density and the capacity for adequate water absorption of this growing media. (Padhiyar *et al.*, 2017). The two-way analysis of variance tested whether protray and pot mixture influenced the single bud chip bajra napier seedling root growth. The two independents variable protray and pot mixture

combination has the *p* value 0.001, which is less than 0.05. Hence null hypothesis is rejected. It concludes that protray and pot mixtures influence bajra napier seedling growth under a single node bud chip method.

The maximum average plant height (39.2 cm) was recorded in the treatment received 60 cavity protray with 30-day old seedlings grown in a potting mixture of red earth + cocopeat + vermicompost (M_2S_2). The minimum average plant height (16.8 cm) was observed in red earth alone with seedlings that were 30 days old. The treatment combination of different protray cavities and growth media was significant at 5% level (Table 2). This could be attributed to higher nutrient status provided by vermicompost and excellent physical (water retention and aeration) and chemical

Table 1: Effect of protray and pot mixtures on seedling growth parameters of bajra-napier.

Treatment	Germination (per cent)		Number of leaves (cm)		Plant height (cm)		Leaf length (cm)		Leaf width (cm)		Root length (cm)	
	15 DAP	30 DAP	15 DAP	30 DAP	15 DAP	30 DAP	15 DAP	30 DAP	15 DAP	30 DAP	15 DAP	30 DAP
Protray												
M_1	50.15	69.94	1.90	5.64	10.08	25.14	4.49	27.74	1.02	1.59	4.38	23.21
M_2	46.36	83.83	3.10	6.84	12.50	30.12	5.19	31.75	1.21	1.96	5.16	25.34
M_3	32.42	61.66	1.94	5.07	8.45	19.84	4.02	24.35	1.01	1.69	4.50	21.46
Pot mixtures												
S_1	43.69	71.56	1.84	6.06	9.20	22.89	4.41	27.15	1.03	1.60	4.37	22.46
S_2	56.77	83.37	2.94	7.06	12.70	30.98	5.64	33.12	1.38	2.13	5.80	27.68
S_3	39.38	66.53	2.06	5.56	10.12	24.33	4.29	25.47	0.98	1.49	4.31	22.81
S_4	44.74	76.34	2.67	5.89	11.34	26.41	4.84	30.56	1.15	1.87	4.89	25.52
S_5	30.32	61.20	2.06	4.67	8.34	20.55	3.67	23.45	0.89	1.62	4.04	18.24
SE (±)												
Protray	0.24	0.35	0.17	0.22	0.17	0.29	0.38	0.34	0.02	0.02	0.04	0.22
Pot mixtures	0.30	0.45	0.22	0.29	0.22	0.37	0.49	0.43	0.02	0.03	0.05	0.29
Significance (P)												
Protray	**	**	**	**	**	**	**	**	**	**	**	**
Pot mixtures	**	**	**	**	**	**	**	**	**	**	**	**

Table 2: Effect of protray and pot mixtures combination on seedling growth parameters of bajra-napier (Mean values).

Treatment	Germination (per cent)		Number of leaves (cm)		Plant height (cm)		Leaf length (cm)		Leaf width (cm)		Root length (cm)	
	15 DAP	30 DAP	15 DAP	30 DAP	15 DAP	30 DAP	15 DAP	30 DAP	15 DAP	30 DAP	15 DAP	30 DAP
Protray*Pot mixture												
M_1S_1	49.60	70.70	1.50	5.50	9.40	23.80	4.31	27.47	0.84	1.62	3.64	21.95
M_1S_2	64.84	84.20	2.50	6.67	12.47	31.20	5.89	33.90	1.35	2.05	5.78	27.17
M_1S_3	42.90	63.31	1.50	5.84	9.49	23.75	3.89	25.64	0.95	1.27	3.89	23.17
M_1S_4	58.35	75.29	2.50	5.50	10.47	26.55	4.62	30.27	1.08	1.62	4.67	25.28
M_1S_5	35.00	56.17	1.50	4.6	8.56	20.36	3.79	21.44	0.88	1.42	3.96	18.52
M_2S_1	50.88	82.51	2.50	7.17	10.37	26.40	4.90	29.34	1.18	1.80	5.02	23.83
M_2S_2	61.84	94.11	3.84	8.17	15.92	39.12	6.16	38.14	1.54	2.37	6.23	30.87
M_2S_3	45.18	77.46	2.84	6.34	12.34	29.06	4.99	28.78	1.06	1.66	4.68	22.62
M_2S_4	41.70	90.87	2.84	7.00	14.42	31.47	5.85	38.36	1.31	2.08	5.60	28.87
M_2S_5	32.20	74.12	3.50	5.51	9.44	24.50	4.03	26.17	0.97	1.39	4.28	20.55
M_3S_1	30.57	61.47	1.50	5.50	7.84	18.46	4.00	24.65	1.05	1.38	4.47	21.60
M_3S_2	43.62	71.84	2.50	6.34	9.70	22.60	4.80	27.30	1.25	1.97	5.38	25.00
M_3S_3	30.00	58.84	1.84	4.51	8.56	20.15	4.02	22.00	0.90	1.56	4.35	22.34
M_3S_4	34.16	62.85	2.67	5.17	9.13	21.20	4.05	25.04	1.05	1.90	4.38	22.40
M_3S_5	23.75	53.24	1.17	3.84	7.00	16.78	3.17	22.75	0.80	1.62	3.90	15.67
SE (±)	0.54	0.787	0.38	0.49	0.89	0.65	0.08	0.76	0.04	0.06	0.08	0.50
Significance (P)												
Protray*Pot mixtures	**	**	NS	NS	**	**	**	**	**	**	**	**

DAP - Days after planting, ** - means 1% level of significance, NS - Non-significance.

properties (acceptable pH, low electrical conductivity, high CEC) of cocopeat, which would have resulted in higher nutrient uptake (Madhavan and Manivannan, 2007).

The number of leaves was high in 30 days old seedlings grown in the media of red earth + cocopeat + vermicompost with the protray of 60 cavities and the minimum number of leaves were found in red earth alone in 98 cavity protray on the same day of seedlings (Fig 5). The protray and growing medium interaction with dependable variables were not significant at 5 per cent level. More leaves in the seedlings may reflect the root system's earlier growth. This could be attributed to vermicompost's higher nutrient status and the excellent physical (water retention and aeration) and chemical properties (acceptable pH, low electrical conductivity, high CEC) of cocopeat, which might have resulted in increased nutrient supply (Nair and Bharathi, 2015).

The root length was one of the influenced growth parameters with different ages of seedlings and growth media. The 30 days aged seedling has the highest root length of 30.87 cm recorded in 60 cavity protray and Redearth + Cocopeat + Vermicompost (M_2S_2). In contrast, the minimum root length (15.61 cm) was observed with 98 cavity protray + Red earth (M_3S_5) (Fig 6). The treatments were (p = 5%) significantly different from each other (Table 3). This is due to the variation of available nutrients in the selected growth media. To individual transplant seedlings, the potting mixture must be heavy enough to make a compact with roots to avoid damage during transporting, handling and transplanting. Because of the synergistic combination of both factors in improving the physical conditions of the media and nutritional factors, the combined application of coir pith and vermicompost in the treatment had a significant impact on seedling root growth parameter (Vivek and Duraisamy, 2017).

50 cavity protray 60 cavity protray 98 cavity protray

Fig 5: Bajra napier single node bud chip seedling growth and leaves at 15 DAP.

50 cavity protray 60 cavity protray 98 cavity protray

Fig 6: Bajra napier single node bud chip seedling root growth at 15 DAP.

Table 3: Tests of between-subjects effects on root length of single bud bajra-napier seedlings at 15 and 30 DAP.

Source	Dependent variable	Type III sum of squares	df	Mean square	F	Sig.
Corrected Model	Average Root length @ 15 DAP	25.068[a]	14	1.791	75.836	.000
	Average Root length @ 30 DAP	616.315[b]	14	44.022	58.162	.000
Intercept	Average Root length @ 15 DAP	986.076	1	986.076	41763.221	.000
	Average Root length @ 30 DAP	24516.336	1	24516.336	32390.931	.000
Protray	Average Root length @ 15 DAP	5.328	2	2.664	112.838	.000
	Average Root length @ 30 DAP	113.645	2	56.822	75.074	.000
Potmix	Average Root length @ 15 DAP	17.411	4	4.353	184.356	.000
	Average Root length @ 30 DAP	455.193	4	113.798	150.350	.000
Protray * Potmix	Average Root length @ 15 DAP	2.328	8	.291	12.326	.000
	Average Root length @ 30 DAP	47.477	8	5.935	7.841	.000
Error	Average Root length @ 15 DAP	.708	30	.024		
	Average Root length @ 30 DAP	22.707	30	.757		
Total	Average Root length @ 15 DAP	1011.853	45			
	Average Root length @ 30 DAP	25155.358	45			
Corrected total	Average Root length @ 15 DAP	25.776	44			
	Average Root length @ 30 DAP	639.021	44			

a. R Squared = .973 (Adjusted R Squared = .960)

b. R Squared = .964 (Adjusted R Squared = .948)

Table 4: Effect of protay and pot mixture combination on growth parameters of bajra napier seedling under single node chip bud method.

Control variables			Germination % @ 15 DAP	Germination % @ 30 DAP	No. of leaves @ 15 DAP	No. of leaves @ 30 DAP	Plant height @ 15 DAP	Plant height @ 30 DAP	Leaf length @ 15 DAP	Leaf length @ 30 DAP	Leaf width @ 15 DAP	Leaf width @ 30 DAP	Root length @ 15 DAP	Root length@ 30 DAP
	Germination % @ 15 DAP	Correlation	1.000	.745	.595	.556	.717	.774	.793	.764	.781	.780	.831	.757
		Sig. (2-tailed)	.	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
		Df		41	41	41	41	41	41	41	41	41	41	41
	Germination % @ 30 DAP	Correlation		1.000	.707	.740	.890	.905	.909	.922	.805	.830	.848	.813
		Sig. (2-tailed)		.	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
		Df			41	41	41	41	41	41	41	41	41	41
	No. of leaves @ 15 DAP	Correlation			1.000	.519	.657	.707	.648	.611	.612	.607	.601	.632
		Sig. (2-tailed)				.000	.000	.000	.000	.000	.000	.000	.000	.000
		Df				41	41	41	41	41	41	41	41	41
	No. of leaves @ 30 DAP	Correlation				1.000	.701	.693	.700	.632	.649	.606	.690	.643
		Sig. (2-tailed)				.	.000	.000	.000	.000	.000	.000	.000	.000
		Df					41	41	41	41	41	41	41	41
	Plant height @15 DAP	Correlation					1.000	.944	.910	.866	.806	.763	.828	.857
		Sig. (2-tailed)					.	.000	.000	.000	.000	.000	.000	.000
		Df						41	41	41	41	41	41	41
	Plant height @ 30 DAP	Correlation						1.000	.903	.899	.843	.796	.838	.837
		Sig. (2-tailed)							.000	.000	.000	.000	.000	.000
Potmixture		Df							41	41	41	41	41	41
and	Leaf length @ 15 DAP	Correlation							1.000	.890	.871	.828	.930	.886
Protray		Sig. (2-tailed)							.	.000	.000	.000	.000	.000
		df								41	41	41	41	41
	Leaf length @ 30 DAP	Correlation								1.000	.847	.793	.828	.825
		Sig. (2-tailed)								.	.000	.000	.000	.000
		df									41	41	41	41
	Leaf width @ 15 DAP	Correlation									1.000	.750	.901	.835
		Sig. (2-tailed)									.	.000	.000	.000
		df										41	41	41
	Leaf width @ 30 DAP	Correlation										1.000	.802	.724
		Sig. (2-tailed)										.000	.000	.000
		df											41	41
	Root length @ 15 DAP	Correlation											1.000	.846
		Sig.(2-tailed)												.000
		df												41
	Root length @ 30 DAP	Correlation												1.000
		Sig. (2-tailed)												
		df												

A partial correlation was run to determine the relationship between growth variables while controlling by protray (Table 4). The values of rare significant at a level less than the 0.05 set as the criteria for statistical significance with the following hypotheses. There is no effect or significant or equal influence of protray and pot mixtures combination on the growth characteristics of bajra napier single bud chip seedling. There is a high positive correlation between germination and plant height at 30 DAP r=0.905, n=41 p<0.001 by the pot mixtures' controlling factor. There is a high positive correlation between germination and leaf length at 30 DAP r=0.922, n=41 p<0.001 in pot mixtures' controlling factor. There is a high positive correlation between germination and root length at 30 DAP r=0813, n=41 p<0.001 with the controlling factor of pot mixtures.

CONCLUSION

The combined effect of protray in different cavities and potting mixture with various proportions on germination and growth of bajra napier single node bud chip seedling was investigated. The maximum growth parameters such as germination, plant height, no. of leaves, leaf length, leaf width and root length (94.11 cm. 39.12 cm, 8.1, 38.14 cm, 2.37 cm and 30.8 cm) were observed at 30 days aged seedlings with 60 cavity protray with red earth + cocopeat + vermicompost. Hence, 60 cavity protray with red earth + cocopeat + vermicompost was found as suitable protray and growth media to establish single node chip bud seedlings in bajra napier. However, these results would be helpful in mass-scale propagation with double the seedling production in with the same traditional multiplication setts and effective utilization of fodder stalk of bajra napier forage. Further field experiments should be conducted to examine under field conditions to establish bajra napier.

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