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Morpho-Physiological Changes Associated With Improved Sprouting Of Sugarcane Bud Chips Treated With Different Chemicals

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ABSTRACT
A field experiment was conducted to evaluate the efficacy of different chemicals on improved sprouting of sugarcane bud chips. The study consisted of seven treatments (Control/untreated, water soaking for 24 hours, Cow dung slurry soaking for 24 hours, ethrel @100ppm for 24 hours, Calcium chloride @ 2mg lit^{-1} for 15-30 minutes, 2, Chloro ethyl phosphonic acid @5ppm for 15-30 minutes, $CaCl_2$+CEPA @ 2mg lit^{-1} and 5ppm for 15-30 minutes).There were two varieties (2003V46, 2003T121) and three replications laid out in split plot design. Significant variation in morpho-physiological characters *viz*., survival percentage, leaf area, coefficient of velocity of germination, seedling vigour index, total dry matter partitioning, shoot length and root length was observed with different chemical treatments. Between two varieties V_1 (2003T121) showed better results and among the treatments $CaCl_2$+CEPA and water soaking performed well.
Key words: *Sugarcane, budchip, seedling vigour index, survival percentage, dry matter partitioning.*

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INTRODUCTION

Sugarcane is a very important commercial cash crop next to cotton grown between 30° N and 30° S latitudes. About 75 per cent of the world sugar (sucrose) is produced from sugarcane and the other 25 per cent comes from sugar beet. Sugarcane is commercially planted using stalk cuttings or setts. In conventional system prevailing in India, about 6-8 tonnes of seed cane per hectare (nearly 10 per cent of produce) is used as planting material. This method of cultivation is gradually becoming uneconomical, as it accounts for over 20 per cent of the total cost of production besides this large mass of planting material poses a great problem in transport, handling and storage of seed cane and undergoes rapid deterioration and decreased viability of buds.

A viable alternative to reduce the mass and improve the quality of seed cane would be the plant excised auxillary buds of cane stalk called bud chips, which are less bulky, more economical and more easily transportable seed material. Through bud chip method, bud chip raised seedlings shall be transplanted instead of normal sett planting. This concept was evolved over a period of around 60 years. The noted Sugarcane Physiologist, Van Dillewijn. (1952) was first to suggest that a small volume of tissue and a single root primordium adhering to the bud are enough to ensure germination in sugarcane. However, this technology did not scale up to commercial level due to poor survival of bud chips under field conditions. Bud chips consist of lower food reserves (1.2 -1.8g sugar) per bud compared to conventional three budded sett material (6-8 g sugar per bud). The food reserves and moisture content of bud chips deplete in a faster way compared to 2-3 budded setts which reflects in their poor sprouting and early growth. Treatment of bud chips with growth promoting chemicals might hasten up germination related metabolic process (Jain *et al*., 2011). Thus, an experiment was conducted to know the effect of different growth promoting chemicals on faster initiation of shoot, roots, early leaf development with higher photosynthetic rate and activation of essential biochemical reactions pre requisite for the establishment and survival of bud chip plantlets in the soil.

MATERIAL AND METHODS:

The field experiment was carried out at Agricultural Research Station, Perumallapalli, Tirupati, situated at an altitude of 182.90 meters above the mean sea level, 13°N latitude and 29°E longitude. Sugarcane variety 2003T121 (Pre-released cultivar) and 2003V46 (Bharani) were selected for this experiment. The experiment was laid out in a split plot design with two varieties (main plot) and seven treatments (sub plot) replicated thrice.

Different treatments imposed on bud chips of both V_1 (2003T121) and V_2 (2003V46) were as follows. V_1T_1 (Untreated bud chips of 2003T 121),V_1T_2 (Water soaking for 24 hours),V_1T_3 (Cow dung slurry 20% for 24 hours),V_1T_4 (Etherel @100ppm for 24 hours), V_1T_5 ($CaCl_2$ @ 2mg lit^{-1} for 15-30 minutes), V_1T_6 (2, Chloro Ethyl Phosphonic Acid @5ppm for 15-30 minutes), V_1T_7 ($CaCl_2$+CEPA @ 2mg lit^{-1} and 5ppm for 15-30 minutes), V_2T_1 (Untreated bud chips of 2003V46), V_2T_2 (Water soaking for 24 hours),V_2T_3 (Cow dung slurry 20% for 24 hours),V_2T_4 (Etherel @100ppm for 24 hours),V_2T_5 ($CaCl_2$ @ 2mg lit^{-1} for 15-30 minutes), V_2T_6 (2, Chloro Ethyl Phosphonic Acid @5ppm for 15-30 minutes) and V_2T_7 ($CaCl_2$+CEPA @ 2mg lit^{-1} and 5ppm for 15-30 minutes).

The experiment was conducted with 126 protrays wherein three protrays were considered as one replication for each treatment. All together there were two varieties and seven treatments replicated thrice. Each dip of the protray was filled with moist cocopith. The bud chips of 2003V46 (Bharani) and 2003T121 (pre released cultivar) were obtained from Agricultural Research Station, Perumallapalli, Tirupati. After imposing the treatments, the bud chips were planted in portrays. The experiment was maintained up to 45 days after planting in portrays and data from the nursery was collected.

The protrays were maintained weed free up to 45 days after planting through manual weeding. Water was applied with a rose can for every 3 days during the period of experimentation (up to 45 DAP). Sampling was done at 15 days interval. Randomly labelled three plants from each replication and from each treatment were removed along with roots for sampling purpose. Data was recorded on the following parameters.

Germination Percentage:

Germination percentage was calculated as follows.

$$\text{Germination \%} = \frac{\text{Number of bud chips germinated}}{\text{Total number of bud chips kept for germination}} \times 100$$

The same was expressed as survival percentage.

Coefficient of Velocity of Germination (CVG):

The coefficient of velocity of germination was calculated by adopting the formula suggested by Kotowski. (1926)

$$CVG = \frac{N_1+N_2\ldots\ldots\ldots\ldots\ldots N_K}{N_1T_1+N_2T_2+\ldots\ldots\ldots\ldots N_KT_K} \times 100$$

Where, 'N' is the number of germinating seeds within the consecutive intervals of time 'T' and 'T' is the time between beginning of the test and the end of the particular intervals of measurement.

Shoot length (cm)

Shoot length was measured from the base of the plant to tip of the leaf at 15, 30 and 45 DAP and was expressed in centimeters.

Root length (cm)

Root length was measured from the base of the plant to the tip of the longer root at 15, 30 and 45 DAP and was expressed in centimeters.

Leaf area (LA) (cm^2 $plant^{-1}$)

After separation of leaves from the plant, leaf area was estimated using leaf area meter (Li-COR model LI 3000) and expressed as cm^2 $plant^{-1}$.

Fresh weight and dry weight of leaf, root and stem (g $plant^{-1}$)

Samples collected from the protrays were separated in to leaves, stem and root. Their fresh weight was recorded individually. The samples were kept in a hot air oven at 100°C for 15 minutes followed by 80°C for 2 days till they attained constant weights. Dry weights were recorded individually.

Seedling Vigor Index

Seedling vigour index was calculated by using the following formula suggested by Abdul-baki and Anderson (1973).

Vigour index = (shoot length + root length) X germination percentage.

RESULTS

Survival percentage:

Spread of germination time was found to be reduced with different chemicals. Data on survival percentage revealed the influence of different chemical treatments on sugarcane varieties 2003T121 and 2003V46 (table.1). Among all treatments T_2 (water soaking) recorded significantly higher survival (59.11%) compared to T_1 (control) (36.67%) followed by T_4 (ethrel) (50.11%). T_3 (cow dung slurry) recorded significantly least survival percentage (38.44) next to control.

Between the varieties, at 15 DAP no significant difference was observed. However the difference was significant at 30 and 45 DAP. V_1 (2003T121) recorded higher survival percentage at 30 DAP (54.51%) and the same was maintained at 45 DAP. Where as in V_2 (2003V46) survival percentage at 30DAP was 37.62%. Interaction between treatments and varieties showed significant difference at all growth stages. V_1 (2003T121) showed significant difference with T_7 (CEPA+$CaCl_2$) (63.11%) compared to control (51.11%).

Coefficient of velocity of germination:

Data on Coefficient of velocity of germination revealed the influence of different chemical treatments on sugarcane varieties 2003T121, 2003V46 and is presented in table 2.

The results from the study revealed that, up to 15 DAP speed of germination was highest and decreased progressively later on. At 15 DAP T_7 ($CaCl_2$+CEPA) showed highest speed of germination (10.80). However there was no significance differences among all the treatments at all the crop growth stages.

Seedling vigour index

Seedling vigour index was estimated at 15, 30 and 45 DAP for comparative evaluation of V_1 (2003T121) with V_2 (2003V46) (Bharani) (high yielding check). With respect to initial vigour and growth V_1 (2003T121) was found to be superior to V_2 (2003V46). Among the treatments T_2 (water soaking) (1947) recorded significantly higher seedling vigour index followed by T_7 ($CaCl_2$+CEPA) (1645.73) T_4 (ethrel) (1286.82), T_6 (CEPA) (1314.81) and T_5 (1090.79). T_1 (724.15) and T_3 (cow dung slurry) (1007.71) recorded the lowest seedling vigour index values. The interaction effect was found to be significantly high in V_1T_7 (2003T121 with $CaCl_2$+CEPA) (2297.20) and V_1T_2 (2003T121 with water soaking) (2220.00).

Seedling vigour index values depends upon root length, shoot length and germination percentage. V_1 (2003T121) recorded higher germination percentage of 39.9% and 54.51% at 15 and 30 DAP compared V_2 (2003V46) for the same period (22.06 % and 37.62%).

Shoot and root length:

Data on Shoot and root length revealed the influence of different chemical treatments on sugarcane varieties 2003T121, 2003V46 and is presented in figure 1 & 2

Though root length did not differ significantly between varieties shoot length was significantly high for V_1 (2003T121) at 15 and 30 DAP and this might be the reason for more seedling vigour index. Among various treatments, shoot length recorded the highest value in T_7 ($CaCl_2$+CEPA) (11.53cm) followed by T_2 (water soaking) where as root length was highest in T_7 ($CaCl_2$+CEPA) (26.48cm) followed by T_2 (water soaking) (25.60cm). T_3 (cow dung slurry) and T_1 (control) recorded lowest values for both root length and shoot length. When the interaction was studied V_2T_7 (2003V46 with $CaCl_2$+CEPA) followed by V_2T_2 (2003V46 with water soaking) (12.10cm) recorded higher shoot length, while V_1T_2 (2003T121with water soaking) (27.13cm) followed by V_2T_7 (2003V46 with $CaCl_2$+CEPA) (26.53cm) recorded highest root length. V_2T_3 (2003V46 with cow dung slurry) (8.73cm) recorded lowest shoot length where V_2T_1 (2003V46 with control) (10.80cm) recorded lowest root length.

Leaf area

Data on leaf area revealed the influence of different chemical treatments on sugarcane varieties 2003T121, 2003V46 and is presented in table 3.

As for as leaf area is concerned V_1 (2003T121) (80.41cm^2 $plant^{-1}$) was found superior to V_2 (2003V46) (27.34 cm^2 $plant^{-1}$) at all the growth stages (15, 30 and 45). Among the treatments T_2 (water soaking) (75.71cm^2 $plant^{-1}$) followed by T_3 (cow dung slurry) (66.27cm^2 $plant^{-1}$) and T_7 ($CaCl_2$+CEPA) (55.42 cm^2 $plant^{-1}$) recorded significantly highest leaf area compared to control (34.12 cm^2 $plant^{-1}$). The interaction of V_1T_2 (2003T121 with water soaking) (112.54 cm^2 $plant^{-1}$) followed by V_1T_3 (2003T121 with cow dung slurry) (109.19 cm^2 $plant^{-1}$) recorded highest leaf area.

Dry matter partitioning:

No significant difference was observed for fresh weight and dry weight of roots between the varieties. The interaction effect was also found non significant at 45 DAP. However, the treatments differ significantly. The highest fresh weight of root was recorded in T_4 (ethrel) (0.54 g $plant^{-1}$) followed by T_7 ($CaCl_2$+CEPA) (0.53 g $plant^{-1}$), T_5 (calcium chloride) (0.44 g $plant^{-1}$) and T_2 (water soaking) (0.43 g $plant^{-1}$). The lowest root fresh weight was observed in T_1 (control) (1.12 g $plant^{-1}$) and T_3 (cow dung slurry)

(1.40 g plant^{-1}). T_2 (water soaking) (0.08 g plant^{-1}) recorded highest root dry weight followed by T_7 ($CaCl_2$+CEPA) (0.07 g plant^{-1}) at 30 DAP. However at 45 DAP it was found non significant.

Fresh weight and dry weight of shoot and leaves followed a similar trend. V_1 (2003T121) was found to be superior to V_2 (2003V46) on both fresh weight and dry weight of shoot and leaves. Among the treatments T_7 ($CaCl_2$+CEPA) (2.25 and 0.37g plant^{-1}) followed by T_2 (water soaking) (1.63 and 0.35 g plant^{-1}) recorded highest fresh weight and dry weight of shoot and leaves. T_1 (control) (0.54 and 0.12 g plant^{-1}), T_3 (cow dung slurry) (1.26 and 0.19 g plant^{-1}) recorded lowest fresh weight and dry weight of shoot and leaves. Among the interaction V_1T_7 (2003T121 with $CaCl_2$+CEPA) (2.48 and 2.44g plant^{-1}) recorded significantly highest fresh weight of shoot as well as leaves. Whereas V_2T_1 (2003V46 with control) recorded lowest in both. At 45 DAS V_2T_7 (2003V46 with $CaCl_2$+CEPA) (0.32 g plant^{-1}) recorded highest shoot dry weight where as V_2T_2 (2003V46 with water soaking) (0.45 g plant^{-1}) recorded highest dry weight of leaves.

DISCUSSION AND CONCLUSION

Morpho-physiological and growth attributes were affected by different chemical treatments. Between varieties V_1 (2003T121) recorded more survival percentage of 54.51% compared to V_2 (2003V46) (37.62%). The treatments also differed significantly with respect to manipulation of germination. T_2 (water soaking) recorded highest germination percentage (59.11%) where as lowest was observed in T_1 (control) (36.67%) and T_3 (cow dung slurry) (38.44%). Among the interactions V_1T_7 (2003T121 with $CaCl_2$+CEPA) (63.11%) recorded highest germination percentage. Spread of germination was observed to be more in V_1 (2003T121) compared to V2 (2003V46).

Water soaking showed better results in survival percentage. Water content of the bud chips was the most important factor for germination related metabolic activity. High percentage of water content in cane favourably influenced germination of bud chips. Optimum level of moisture for sugarcane germination is 50.3% (Panje and Gill, 1962).

Different morphological attributes *viz*., shoot length, root length, total dry matter, number of leaves and leaf area was found to be the highest in T_2 (water soaking) and T_7 ($CaCl_2$+CEPA)followed by T_5 (calcium chloride), T_6 (CEPA) and T_4 (Ethrel). It might be due to the influence of CEPA and Calcium chloride on the improved activity of acid invertase and ATP ase enzymes. Acid invertase hydrolyses sucrose in to hexoses and ATPase liberates inorganic phosphorus to provide cells with carbon and energy for synthesis of different compounds essential for sprouting and subsequent growth.

CEPA an ethylene generating compound is a versatile growth regulator. Ethylene has been implicated as a factor that controls the timing of seed germination rate and dimensions of etiolated seedlings, growth and leaf expansion, initiation and progression of abscission and fruit ripening and the expression of number of stress related responses in plants (Anthony and Schaller, 1996: Cassab *et al*., 1998). In sugarcane the growth stimulating effects of ethephon and calcium chloride have been demonstrated on sprouting of aerial and underground stubble buds of winter initiated ratoons (Solomon *et al*., 1998: Jain *et al*., 2009).Calcium is an essential plant nutrient which stimulate the growth and development of plants. Its structural role in the cell wall, membranes and in the vacuole as a counter cation for inorganic and organic anions and as an intra cellular messenger in the cytosol was explained (Burstrom, 1968; white and Broadly, 2003). It plays an important role in various physiological processes by acting as a second messenger in the transduction of endogenous and exogenous signals (Helper and Wayne, 1985). It is necessary for cell elongation and known to activate a number of enzymes such as a phospholipase-D, lecithinase, ATP ase and amylase (Davidson and Long, 1958; Kalcker, 1944; Chrispeels and Varner, 1967; Helper, 2005).

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Figure: 1 Effect of different chemical treatments on shoot length (cm) of bud chip seedlings.

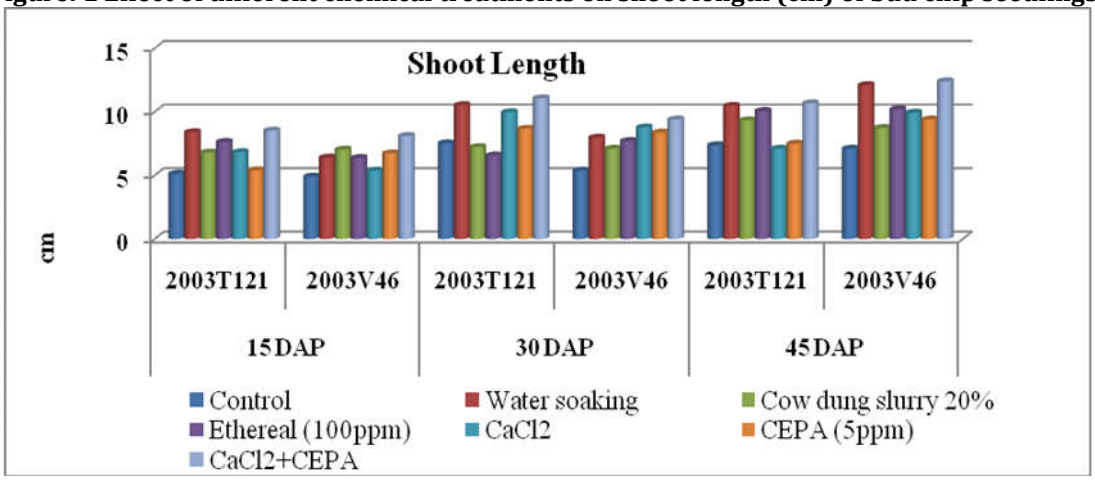

Figure: 2 Effect of different chemical treatments on root length (cm) of bud chip seedlings.

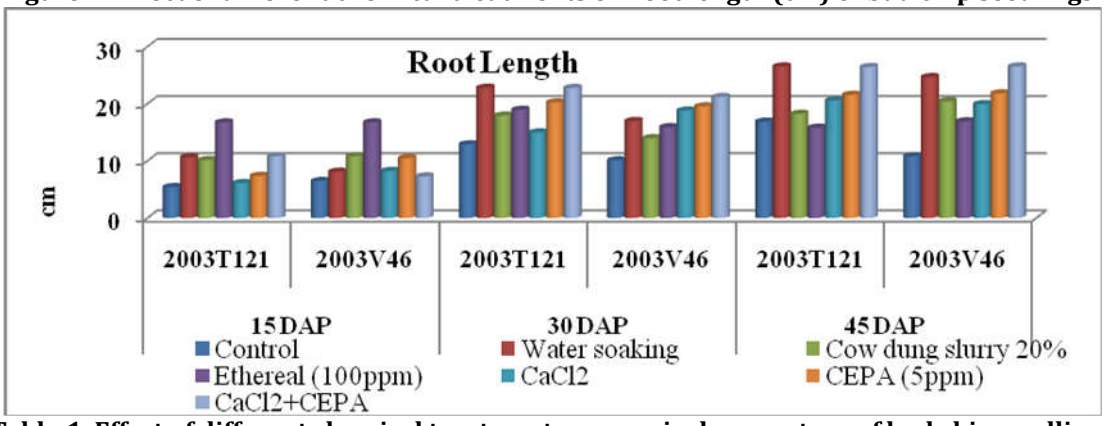

Table:1 Effect of different chemical treatments on survival percentage of bud chip seedlings

		15 DAP			30 DAP			45 DAP		
	Treatments	**V1**	**V2**	**MEAN**	**V1**	**V2**	**MEAN**	**V1**	**V2**	**MEAN**
T_1	Control	33.56	4.44	**19.00**	51.11	22.22	**36.67**	51.11	22.22	**36.67**
T_2	Water soaking	52.44	34.45	**43.45**	61.56	56.67	**59.11**	61.56	56.67	**59.11**
T_3	Cow dung slurry 20%	30.00	17.78	**23.89**	44.67	32.22	**38.44**	44.67	32.22	**38.44**
T_4	Ethrel (100ppm)	43.11	36.67	**39.89**	59.11	41.11	**50.11**	59.11	41.11	**50.11**
T_5	$CaCl_2$	31.32	26.67	**29.00**	44.00	41.11	**42.56**	44.00	41.11	**42.56**
T_6	CEPA (5ppm)	39.33	15.56	**27.45**	58.00	41.11	**49.56**	58.00	41.11	**49.56**
T_7	$CaCl_2$+CEPA	49.56	18.89	**34.22**	63.11	28.89	**46.00**	63.11	28.89	**46.00**
	MEAN	**39.90**	**22.06**		**54.51**	**37.62**		**54.51**	**37.62**	
		V	**T**	**V x T**	**V**	**T**	**V x T**	**V**	**T**	**V x T**
	C.D (P= 0.05)	**NS**	**9.31**	**13.16**	**14.07**	**9.36**	**13.23**	**14.07**	**9.36**	**13.23**
	S.Em.±	**4.34**	**3.18**	**4.50**	**2.31**	**3.2**	**2.06**	**2.31**	**3.2**	**2.06**

Table: 2 Effect of different chemical treatments on coefficient of velocity of germination of bud chip seedlings.

		15 DAP			30 DAP			45 DAP		
	Treatments	**V1**	**V2**	**MEAN**	**V1**	**V2**	**MEAN**	**V1**	**V2**	**MEAN**
T_1	Control	10.33	8.33	9.33	5.52	4.28	4.90	5.52	4.28	4.90
T_2	Water soaking	10.39	10.94	10.66	6.48	5.84	6.16	6.48	5.84	6.16
T_3	Cow dung slurry 20%	9.59	10.75	10.17	5.34	5.61	5.47	5.34	5.61	5.47
T_4	Ethrel (100ppm)	9.70	9.95	9.82	6.41	6.07	6.24	6.41	6.07	6.24
T_5	$CaCl_2$	10.15	10.06	10.11	6.22	6.38	6.30	6.22	6.38	6.30
T_6	CEPA (5ppm)	11.45	9.76	10.60	6.54	5.05	5.80	6.54	5.05	5.80
T_7	$CaCl_2$+CEPA	11.99	9.62	10.80	6.30	5.91	6.11	6.30	5.91	6.11
	MEAN	10.51	9.92		6.11	5.59		6.11	5.59	
		V	T	V x T	V	T	V x T	V	T	V x T
	C.D (P= 0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS
	S.Em.±	0.21	0.53	0.75	0.42	0.52	0.74	0.42	0.52	0.74

Table: 3 Effect of different chemical treatments on leaf area (cm^2 $plant^{-1}$) of bud chip seedlings.

		15 DAP			30 DAP			45 DAP		
	Treatments	**V1**	**V2**	**MEAN**	**V1**	**V2**	**MEAN**	**V1**	**V2**	**MEAN**
T_1	Control	17.45	6.77	12.11	39.94	8.12	24.03	53.07	15.17	34.12
T_2	Water soaking	35.63	22.84	29.24	60.50	25.38	42.94	112.54	38.87	75.71
T_3	Cow dung slurry 20%	13.90	11.17	12.53	52.79	12.24	32.52	109.19	23.35	66.27
T_4	Ethrel (100ppm)	29.28	11.67	20.47	58.37	19.29	38.83	62.40	25.82	44.11
T_5	$CaCl_2$	10.64	18.08	14.36	37.68	12.43	25.06	74.44	23.52	48.98
T_6	CEPA (5ppm)	23.56	18.78	21.17	51.55	20.13	35.84	82.25	22.77	52.51
T_7	$CaCl_2$+CEPA	43.15	19.47	31.31	57.17	22.16	39.67	68.99	41.86	55.42
	MEAN	**24.80**	**15.54**		**51.14**	**17.11**		**80.41**	**27.34**	
		V	**T**	**V x T**	**V**	**T**	**V x T**	**V**	**T**	**V x T**
	C.D (P= 0.05)	**2.51**	**2.50**	**3.54**	**2.81**	**4.7**	**6.65**	**8.66**	**7.06**	**9.99**
	S.Em.±	**0.41**	**0.86**	**1.21**	**0.46**	**1.61**	**2.28**	**1.42**	**2.42**	**3.42**

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