Pak. J. Biotechnol. Vol. 12 (2) 161- 168 (2015)
www.pjbt.org
ISSN Print: 1812-1837
ISSN Online: 2312-7791

EXPLORATION OF SOMACLONAL VARIATIONS IN ELITE THREE SUGARCANE GENOTYPES

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Article Received August 10, 2015, Revised October 04, 2015, Accepted November 16, 2015

ABSTRACT:

Sugarcane is commercially important and major cash crop of Pakistan. According to the increasing demand of sugarcane somaclonal variation play vital role to overcome problems for sugarcane breeding. The aim of this study to evaluate genetic variation developed through soma clones and for this purpose three sugarcane genotypes were optimized under *in- vitro* conditions. In parents all parameters except treatment X concentration and concentration X variety was non-significant for height of cane, number of tillers and width of internodes. In soma clones treatment X concentration was non-significant for number of tillers, internode and width of internodes. As compare this effect of growth hormones and varieties X concentration at width of internodes was again non-significant at $p < (0.05)$ vice versa. According to mean comparison at $p < (0.05)$ LSD revealed all the auxins used positively stimulated number of tillers, height of cane, number of internodes and width of internodes. Somaclonal variation is good source of creating genetic variability in the genotypes of sugarcane and developing new varieties. Maximum genetic improvement was induced in NIA-2012. Minimum genetic improvement was induced in Gulabi- 95. Application of 2, 4-D and picloram were efficient for achieving higher, phenotypical trait, cytological and economic traits of sugarcane soma clones.

Key words: Sugar cane, Somaclonal, Genetic variation

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is a monocot-cotyledon crop growing in tropic and subtropics area (Shah *et al.,* 2009) and plant belongs to the family Gramineae (Sharma, 2005; Chaum *et al*., 2006). It has chromosome number of 2n =80 (Daniels and Roach, 1987; Asano *et al.,* 2004). Pakistan occupies fifth position in cane acreage and almost 15th position in sugar production. Sugarcane yield have increased from 36.5 to 43.4 tone's/ha for as a whole (Hussain et al., 2012), yet it is insufficient to the demand of Pakistan. Improvement in sugarcane crop inside different countries depends on conventional breeding, mutation breeding, somaclonal variation and genetic engineering (Dalvi *et al*., 2012, Rajeswari *et al*., 2009). In Pakistan sugarcane development is time consuming because the climatic conditions are not feasible for cane production (Shahid *et al.,* 2011). Sugarcane tillers at the base, grows four to five meters height and about four and half cm in width (Singh, 2003). So, there is a dire need for the applications of modern biotechnological approaches such as the development of transgenic Plants and exploitta-tion of somaclonal variation to improve sugarcane clones for desirable traits (Karim *et al.*, 2015).

The genetic variations can be improved by increasing duration and number of subcultures, preexisting mutations can be enhanced by cultural conditions especially different concentration of growth hormone. Alteration in genetic mechanism includes altered DNA replication and repair of DNA segments which leads to high level of chromosomal aberrations. While epigenetic level includes changes in methylation (Leljak-Levanic *et al*., 2004) and histone modifica-tions (Li *et al*., 2005). The chromosome number and ploidy levels in some plants have been reported to be affected by the type, concentration and combination of synthetic analogues of auxins and cytokinins (Bairu *et al*., 2011). Rapid in-vitro multiplications of the elite sugarcane clones as well as for the improvements of epigenetic, genetic and physiological

(Rastogi *et al*., 2015) of characters through somaclonal variation (Nighat *et al*., 2011; Leal *et al*., 1996; Bahera *et al*., 2009) for the fulfillments of recent requirement of sugarcane (Karim *et al.,* 2015) need continuous attention. Present study helpful for exploitation of somaclonal variation which affected through different auxin concentration. Improvement of desire phenotypic traits possible through soma clones instead of using mutation.

MATERIALS AND METHODS

MS modified with three auxins through varied concentration for callus formation fifteen media preparations were applied. After two subcultures (4 weeks of each), calli were transferred in bottles containing the medium of regeneration (MS modified indole-3-acetic acid, indole-3-butyric acid and kinetin 2.00, 2.50 and 3.0mg L^{-1} of each growth regulators. Cultures were incubated in growth room with (2000-3000 lux) under 16th photo period 25±2^{0}C. The effects of callus age on regeneration were predictable by transferring the calli to regeneration media after 12, 16, 18.25 days of culture. The mass of regenerable calli produced on the five callus induction media were transferred to 5 types of regeneration media. Shoot regeneration started with the appearance of green dots on callus within two weeks on regeneration medium and generally produced normal shootings. No response was observed on control or hormone free MS medium.

Hardening of regenerated plantlets in the pot house: In vitro regenerated plantlets of three soma clones (NIA-2012, NIA-105 and Gulabi-95) were taken out from bottles and were washed under running tap water till unnecessary callus cells (non regenerable) were removed. The plantlets were shifted in to jiffy pots containing soil and organic manure (3:1). The plantlets were cove red with polythene covers to maintain humidity.

Transfer to the field: The plantlets were shifted in large clay pots containing same ratio of soil and organic manure (3:1) after 12-15 days. The pots were watered regularly. Four hundred and five soma clones were transferred from pots to the field in randomized complete block design, and replicated three times.

Observation of phenotypic characters: Only 135 stable soma clones were finally evaluated for four phenotypic characters related to yield.

Number of tillers (plant^{-1}): Three randomly selected canes were counted for number of tillers from each treatment. Thereafter, their average was noted.

Height of cane (cm): The height of each selected plant was measured in centimeters from the surface of soil to the tip of the leaf

Width of internode (cm): The stem girth of eah plant was measured in centimeters by vernier caliper from bottom, mid and top portion and average of the three data was used for data analy sis.

Number of Internodes (plant^{-1}): Three randomly selected canes from each treatment were counted for number of internode. Thereafter, their average was noted.

Statistical analysis: Means of somaclonal variation were subjected to analysis of variance (ANOVA according at 0.01, 0.05 and 0.001 probability levels by computer software Statisix 8.1. All the means of genetic parameters were compared by standard error (SED) and Least Signficant Difference (LSD) using the following formula: T (0.05) df = value form the t distribution table at 5 % probability level and error degree of freedom. Significant levels are shown in the tables for 0.05%, and 0.01% probability levels respectively.

$$LSD = SED \times t\ (0.05)\ df$$

Where EMS = error mean square

RESULTS AND DISCUSIONS

ANOVA for Phenotypic characters of soma clones: The main parameters of external appearance in the sugarcane plant are number of tillers, height of cane as they direct effect on final phenotypic traits in sugarcane. The results of ANOVA for phenotypic characters are presented in Table-1a. In parents highly significant variation was observed for all the parameters except treatment X variety non-significant for height of cane and number of tillers. Whereas concentration X variety was again non-significant for number of tillers. In soma clones only effect of concentration on height of cane and effect of treatment X variety on number of tillers was non-significant $p < (0.05)$ and for rest of phenotypic characters are vice versa.

The results of ANOVA for economic traits are compiled in Table-1b. In parents highly significant variation was observed for all the parameters except treatment X concentration and

concentration X variety was non-significant for width of internode. In soma clones treatment X concentration was non-significant for number of internode and width of internode. According to the results the effect of variety X concentration on width of internode was again non-significant at $p < (0.05)$.

Analysis of variance for number of tillers and height of cane indicated the presence of enough variability in soma clonal population (Table-1a and 1b). The number of internode and width of internode exhibited wider variation in all the soma-clones and the heterotic effect was marked. Present results are in agreement with the earlier findings of Khan *et al*., (2004, 2007), Begum *et al.,* (2011), Raza *et al*., (2014) and Seema *et al*., (2014). All of these reported somaclonal variation as an important tool for induced varition during in vitro cultures.

Table-1a: ANOVA for number of tillers and height of cane modulated by different concentration of growth hormones.

Source	D.F	Mean S quare			
		Height of cane		Number of tillers	
		Soma clones	Parents	Soma clones	Parents
Replication	2	7455.45	60.108	5.4889	5.0296
Treatment	2	6957.65*	10.084**	6.7556*	2.3407 *
Concentrations	4	6662.07ns	411.515**	26.3704**	12.1593**
VAR	2	4570.04*	387.740*	78.6889 **	67.0296**
T x C	8	7564.78*	1.344*	4.7926 **	4.5537**
T x VAR	4	7455.44*	4.553ns	2.7444ns	2.4074ns
C x VAR	8	7577.19*	12.591**	4.8926*	2.0204ns
Error	104	7347.88	5.116	1.5595	1.5799
Total	134	CV 68.84	CV 1.92	CV 23.42	CV 27.24

In each column, means followed by common letter are not significantly different at 5% probability level.

Table-1b:ANOVA for number of internodes and width of internodes modulated by different concentration of growth hormones.

Source	D	Mean square			
		Number of internodes		Width of internodes	
		Soma clones	Parents	Soma clones	Parents
Replication	2	73.489	82.689	0.61051	0.58266
Treatment	2	58.867**	79.489**	0.15736**	0.16620**
Concentration	4	271.567**	193.859**	1.17002**	1.10419**
VAR	2	108.067**	34.022**	1.66817**	1.39556**
T x C	8	2.672ns	1.387 *	0.00869ns	0.00655ns
T x VAR	4	6.433ns	13.744**	0.02932*	0.02904*
C x VAR	8	7.928**	5.337**	0.03589*	0.02911ns
Error	104	3.589	4.767	0.02790	0.02682
Total	134	CV 11.20	CV 14.00	CV 10.61	CV 10.67

In each column, means followed by common letter are not significantly different at 5% probability level.

Mean performance of soma clones in NIA-2012, NIA-105 and Gulabi-95: The result of mean comparison of soma clones and their parents for phenotypic character are compiled in (Table 2a and figure 1a, 1b, 2a & 2b).The pooled performance of soma clones and their parents was significant at ($p<0.05$) in all genotypes for most of economic traits. Maximum number of tillers were found at 2mg/l of 2,4-D (7.33) and minimum at 3mg/l in NAA (5.66). Maximum height of cane was obtained 3mg/l 2,4-D (126.47) and minimum height of cane at 3mg/l of NAA (123.85). Maximum number of internode at 3mg/l of 2,4-D (24.88) and minimum number of internode at 3mg/l of NAA (21. 33). Maximum width of internode observed in 2, 4-D (2.17) followed by picloram (2.09) and minimum width of internode was found in NAA (1.95).

The data of NIA-105 soma clones and their parents for phenotypic character are presented in (Table 2b, Figure 3a,3b,4a & 4b). Maximum number of tillers were found at 3mg/l of 2,4-D (6.11) and minimum at 3mg/l in NAA (5.22). Maximum height of cane was obtained 3mg/l picloram (123. 79) and minimum height of cane at 3mg/l of NAA (122.17). Maximum number of internode at 3mg /l of picloram (20.111) and minimum number of internode was found at 3mg/l of NAA (18.11). Maximum width of inter-node observed in NAA (1.84) followed by picloram (1.59) and minimum width of internode was found in 2, 4-D (1.58).

Table-2a: Impact of different auxins on NIA-2012 soma clones for phenotypic trait development.

Growth regulator	Phenotypic character	Growth regulators Concentrations (mg l^{-1})				
		Control (0)	0.5	1.0	2.0	3.0
2,4-D	Number of Tillers	4.77 e-f	6.55 a-c	7.44 a	7.33 a	7.11 a-b
	Height of Cane	114.1 e-g	117.4c-d	119.2 c	123.05 b	126.4 a
	Number of Internode	13.77 g- h	16.55 d-e	17.88 c-d	20.22 b	24.88 a
	Width of Internode	1.50 e-g	1.60 d-e	1.71 c-d	1.88 b	2.17 a
Picloram	Number of Tillers	4.33 d-g	5.00 c-e	6.33 a-b	5.77 a-c	6.77 a
	Height of Cane	113.4 b	116.76 b	118.2 b	122.08 b	125.2 b
	Number of Internode	12.66 f-g	14.88 d-e	15.66 d	18.11 b-c	21.66 a
	Width of Internode	1.446 d-e	1.55 c-d	1.66 b-c	1.81 b	2.09 a
NAA	Number of Tillers	4.77 a-c	4.333 a-c	5.88 a	5.22 a-c	5.66 a-b
	Height of Cane	116.5 c-d	116.69 c-d	117.7 c-d	121.3 a-b	123.8 a
	Number of Internode	14.44 d-f	16.00c-e	17.11 b-d	18.22 a-c	21.33 a
	Width of Internode	1.3733 d-f	1.4911 c-e	1.611 b-d	1.73 a-c	1.95 a

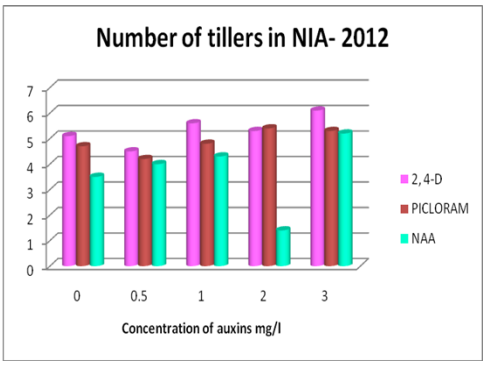

Figure-1a: Effect of 2,4-D on Somaclonal variation for number of tillers in NIA-2012

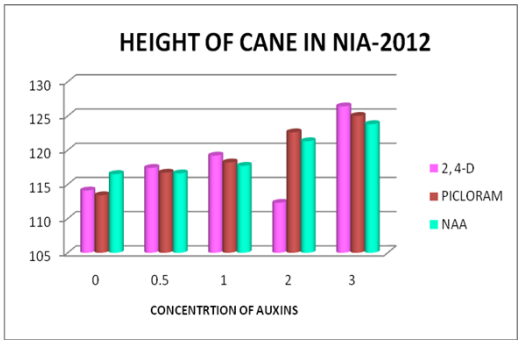

Figure-1b: Effect of 2,4-D on Somaclonal variation for height of cane in NIA-2012

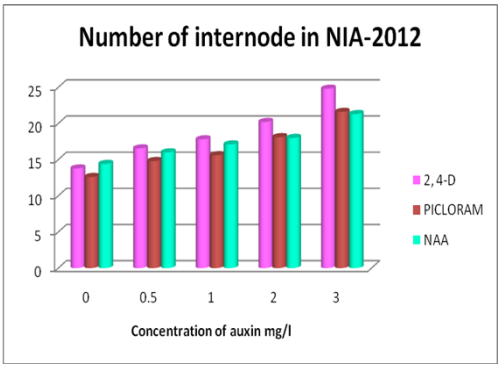

Figure- 2a: Effect of 2,4-D on Somaclonal variation for number of internodes in NIA-2012

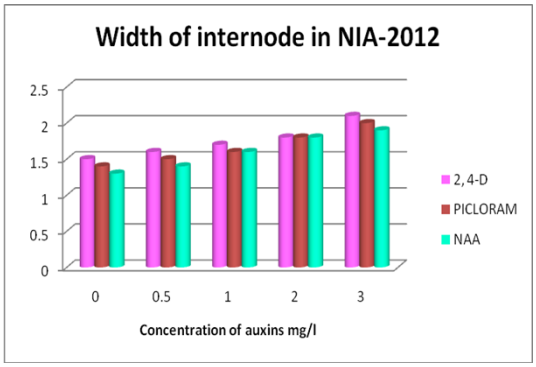

Figure -2b: Effect of 2,4-D on Somaclonal variation for width of internodes in NIA-2012

Table-2b: Impact of different auxins on NIA-105 soma clones for phenotypic trait development

Growth regulators	Phenotypic character	Growth regulators Concentrations (mg l^{-1})				
		Control (0)	0.5	1.0	2.0	3.0
2,4-D	Number of Tillers	5.11 d-f	4.55 e-f	5.66c-e	5.33d-f	6.11 b-d
	Height of Cane	113.6 f-g	115.5 d-f	118.93 c	122.4 b	123.2 b
	Number of Internode	13.00 e-f	14.00 d-f	14.00d-f	17.11b-d	19.77 a-b
	Width of Internode	1.1600 g	1.25f-g	1.37 e-f	1.46d-e	1.55 c-d
Picloram	Number of Tillers	4.77 c-f	4.22 e-g	4.88 c-e	5.44b-c	5.33 b-e
	Height of Cane	112.7 b	115.4 b	118.62 b	121.21 b	123.7 b
	Number of Internode	12.66 g-h	15.55 e-f	15.77 e-f	17.55c-d	20.11 b
	Width of Internode	1.195 i	1.28 h-i	1.428 f-h	1.48e-g	1.59 d-e
NAA	Number of Tillers	3.55 b-c	4.00a –c	4.33 a-c	4.33 a-c	5.22 a-c
	Height of Cane	114.41 d-f	114.4 d-f	115.7 d-e	119.76 b-c	122.17 a-b
	Number of Internode	12.000g	13.4 e-g	15.22 d-e	16.33 b-d	18.11 b-c
	Width of Internode	1.3022 e-f	1.4322d-f	1.5567c-d	1.69 b-c	1.84 a-b

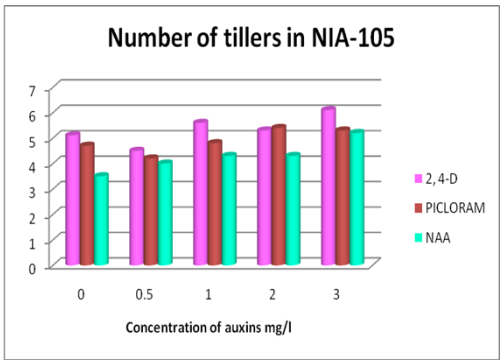

Figure- 3a: Effect of 2,4-D on Somaclonal variation for number of tillers in NIA-105

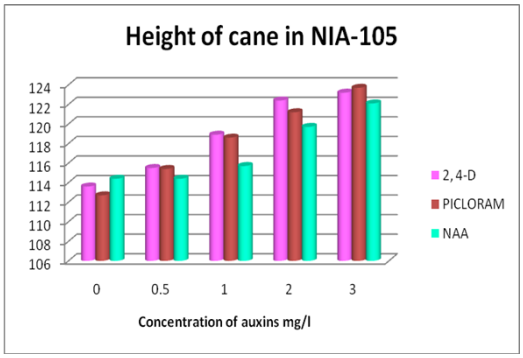

Figure-3b: Effect of 2,4-D on Somaclonal variation for height of cane in NIA-105

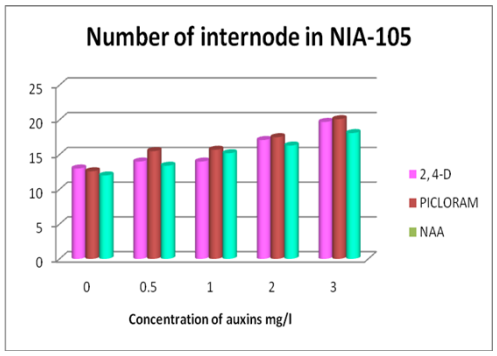

Figure-4a: Effect of picloram on Somaclonal variation for number of internodes in NIA-105

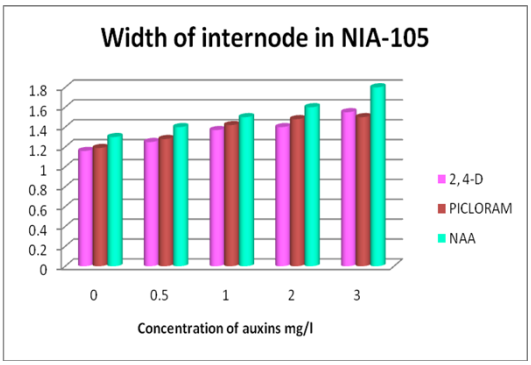

Figure-4b: Effect of NAA on Somaclonal variation for width of internodes in NIA-105

The results of Gulabi- 95 soma clones and their parents for phenotypic characters are organized in (Table 2c, Figure 5a, 5b, 6a and 6b). In case of Gulabi-95 maximum number of tillers was recorded at 3mg/l 2, 4-D (6.11) and minimum number of tillers at 3mg/l of NAA (4.22). Maximum height of cane at 3mg/l of 2, 4-D (223.4) and minimum height of cane at 3mg/l of NAA (119.68). Maximum number of internodes in 2, 4-D (19.22) minimum number of internode was recorded at 3mg/l of picloram (18.38). Maximum width of internode at 3mg/l of picloram (1.8) and minimum width of internode at 3mg/l of NAA (1.62).

Table -2C: Impact of different auxins on Gulabi-95 soma clones for phenotypic trait development.

Growth regulators	Phenotypic character	Growth regulators Concentrations (mg l^{-1})				
		Control (0)	0.5	1.0	2.0	3.0
2,4-D	Number of Tillers	2.00 g	3.0000 g	4.3333 f	4.55 e-f	6.11 b-d
	Height of Cane	110.5 b	114.12 b	117.31 b	115.28 b	223.40 a
	Number of Internode	12.44 h	14.22 f-g	15.55 e-f	17.22 d-e	19.22 b-c
	Width of Internode	1.25 f-g	1.411 d-e	1.53 c-d	1.66 b-c	1.77 b
Picloram	Number of Tillers	5.00 a-c	3.22 c	3.22 c	5.44 a-b	4.44 a-c
	Height of cane	111.05 h	112.8g-h	114.8 e-g	116.2 d-e	118.38 c
	Number of internode	12.11 g	14.66 d-f	14.66 d-f	16.11 c-d	18.33 b
	Width of internode	1.28 h-i	1.396 g-h	1.563 d-f	1.68 c-d	1.80 b-c
NAA	Number of tillers	2.33 i	2.666h-i	3.66 f-h	3.44 g-i	4.22 e-g
	Height of cane	111.5 f	112.4 e-f	112.81 e-f	117.44 c-d	119.68 b-c
	Number of internode	12.11 f	12.11 f	12.55 e-f	15.22 c-f	17.00 b-d
	Width of internode	1.21 f	1.27 e-f	1.41 d-f	1.506 c-e	1.62 b-d

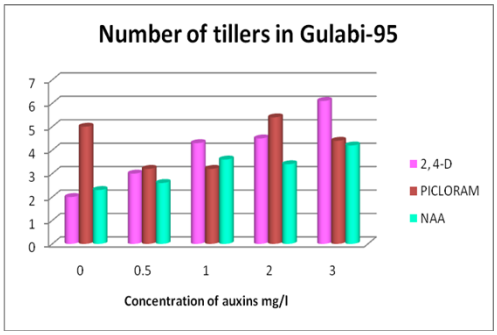

Figure-5a: Effect of 2,4-D on Somaclonal variation for number of tillers in Gulabi-95

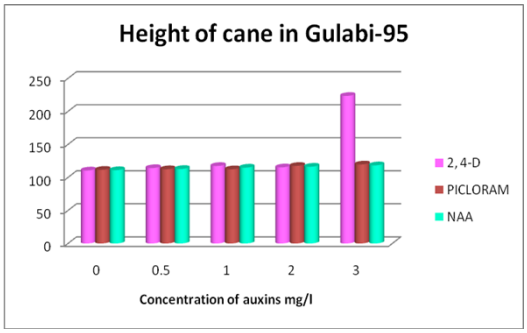

Figure-5b: Effect of 2,4-D on Somaclonal variation for height of cane in Gulabi-95.

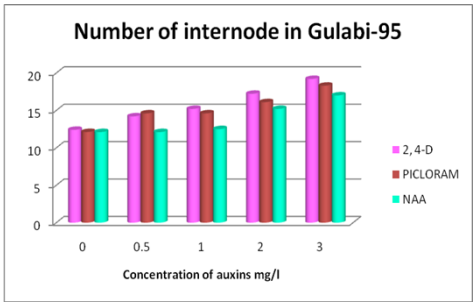

Figure- 6a: Effect of 2,4-D on Somaclonal variation for number of internodes in Gulabi-95

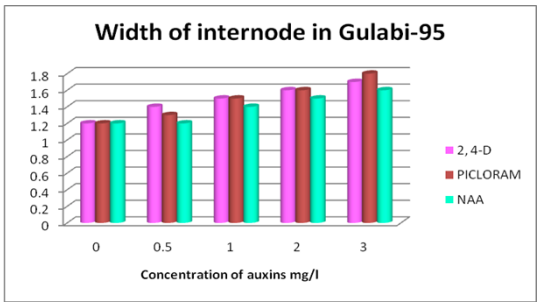

Figure-6b: Effect of picloram on Somaclonal variation for width of internode in Gulabi-95.

It is evident from the result (Table 2a) that all the auxins were used stimulated positively to mean number of tillers, height of cane, number of internode and width of internode. Present results are consisted with the finding of Khan *et al*., (2009), Nawaz *et al*., (2013), Simiar *et al.,* (2014) and Rastogi *et al*., (2015) Additive effects of auxins were observed for all the phenotypic traits. 2, 4-D for NIA-2012 responded best as compare to the other growth hormones. Shahid *et al*., (2012) reported 2, 4-D as best auxin for inducing variability in the phenotypic chracter of sugarcane.

Present results depicted that height of cane and number of internode increases with increase in concentration of picloram applied (Table 2b). All the treatments gave best results at 3.0mg/l in NIA-105. Goel *et al*., (2011) found best results was obtained at lower concentration of picloram, where-

as Present results are in agreement with the finding of Pandey *et al.,* (2012).

It is clear from the result (Figure-7,8,9 and 10) that soma clone of Gulabi -95 showed not more variability in the phenotypic character as compare to the other clones of varieties (Table 2c). 2, 4-D and picloram performed better than NAA. Width of internode depends upon the application of all three auxins. Present results are similar with the finding of Baksha *et al.,* (2002), Khatun *et al*., (2003) and Sabaz *et al.,* (2008). Feyissa *et al*., (2014) reported lowest concentration of NAA was better than increasing concentration.

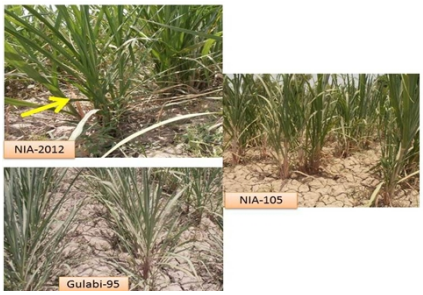

Figure-7: Phenotypic variation induced by auxins for number of tillers among three sugarcane varieties.

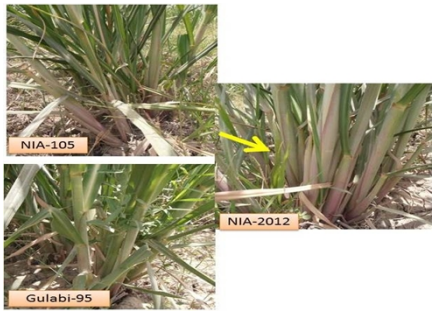

Figure- 8: Phenotypic variation induced by auxins for number of internode among three sugar cane varieties.

Figure-9: Phenotypic variation induced by auxins for width of internode among three sugarcane varieties.

Figure-10: Phenotypic variation induced by auxins for height of cane among three sugarcane varieties

CONCLUSIONS

As concentrations of Auxin are positively associated with level of induced genetic variability and their effect on the heritability of sugarcane is variety dependent, therefore it is suggested that standardization of protocol for type and concentration of auxins must be made for each genotype separately. Somaclonal variations are ideal in vitro techniques to improve the efficiency of breeding programs.

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