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Effect of harmones on callus induction in Maize (*Zea mays L.*)

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Abstract: Callus induction from explants is a critical process in regeneration, micropropagation and transformation of maize (*Zea mays L.*) plants. Formation of callus from plant tissues on culture is affected by several factors. This study revealed to establish the effect of genotype, source of explants and auxin concentration on callus induction from five genotypes UMI 757 (G1), UMI 615 (G_2), UMI 112 (G_3), UMI 285 (G_4) and CO 1 (G_5) and one hybrid CO H (M) 5 (G_6). Callus induction of the six maize varieties was investigated using immature embryos (E_1), leaf bits (E_2), root tips (E_3), hypocotyls (E_4) and seeds (E_5) as explants with different concentrations of hormones. In this study, immature embryo was taken from 10 to 12 days after pollination (DAP) to get maximum response. The highest percentage of callus induction was observed (99.10) in immature embryo culture and seed culture gave the highest percentage of rhizogenic callus formation when compare to immature embryo. Among the genotypes tested, CO H (M) 5 recorded the highest callus induction percentage on (2D2K2) medium composition.

Keywords: Callus induction, Hormones, Immature embryo, Maize, Seed.

INTRODUCTION

Maize (*Zea mays L.*) with 2n = 20, is the third most widely distributed crop of the world after Rice and Wheat (Devi, *et al.,* 2016) being grown in diverse seasons and ecologies with highest production and productivity among food cereals. It is an important source of carbohydrate, protein, iron, vitamin B and minerals (P and K). In Sudan, maize is produced using traditional or mechanical methods and is mainly used for food, forage and is a potential source of foreign exchange through export (Omer *et al.,* 2008). Maize production is affected by biotic and a biotic factors. There constraints can be overcome through development of varieties that can tolerate (or) resist the stress. This can be done through complementing conventional breeding and genetic transformation. Success in plant transformation is dependent on the ability to regenerate a whole plant from transformed tissue (Ahmadabadi *et al.,* 2007) plant regeneration through tissue culture of maize was first reported by Green and Philips (1975). *Agrobacterium* mediated transformation of maize needs efficient regeneration systems. In this study, six maize genotypes were evaluated for their response to tissue culture at different hormone concentrations using five different explants sources.

MATERIALS AND METHODS

Plant Materials: Seeds of maize (*Zea mays L.*) genotypes, UMI 757, UMI 615, UMI 112, UMI 285 and CO 1 and one hybrid CO H (M) 5 were used in this study. These seeds were surface sterilized with 70% ethanol for 2 minutes followed by 0.1% $HgCl_2$ for 5 minutes and then washed with three to four times with sterilized distilled H_2O under aseptic conditions. The sterile seeds were used as source of explants for callus induction. To establish plants to obtain leaf bits, root tip, Hypocotyl explants, sterile seeds were planted in sterile Jam jars containing MS basal salts (Murashige and Skoog, 1962). The Jar were kept in a growth room and maintained at a temperature of 28ºC and sterile seeds. Immature embryo also used as explants. Immature embryos of 1.0- 2.0 mm size were aseptically excised from surface sterilised kernels. The Immature embryos were placed on the semisolid Ms medium with the rounded scutellar side exposed and the plant Plumule radical axis side in contact with the medium.

Preparation of callus induction: Callus induction were performed on MS medium comprising of MS salts and vitamins supplemented with Macronutrients, Micronutrients, Micronutrient and Vitamins, 3% (w/v) sucrose. The pH of the medium was adjusted to 5.6 to 5.8 with IM NaoH or 0.1 M HCl and 0.8% (w/v) agar added before autoclaving to sterilize. The sterilized medium was allowed to cool before adding 2.4-D. The medium was dispensed in sterile Petridishes in volumes of 30 ml and allowed to solidity. Explants were cultured on the medium and plates sealed with parafilm. Sixteen levels of treatment combinations of 2,4-D and kinetin were tested to establish their efficacy in

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establishing callus from five different explants. The mature and healthy seeds of maize were taken for callus Induction. The seeds were sterilized with 70% ethanol for 2 min followed by 0.1 percent Hg Cl $_2$ for 5 min. Sterilized seeds were inoculated under aseptic conditions in the callus induction medium. The culture tubes were incubated in darkness at 25 ± 2ºC for callus induction. The Immature embryo has proven to be the best source for the establishment of embryogenic callus and plant regeneration in maize. The Immature Embryo from maize cob were collected 10-16 days after pollination. The kernels were surface sterilized under Laminar Air Flow Chamber, the Immature embryos were aseptically excised from the kernels of the ear by cutting off kernels using scalpel blade and removing the endosperm. The immature embryo was placed on semisolid medium and was incubated for callus induction. About one cm explants were cut from seven day old seedlings collected from aseptically germinated seedlings. Inoculation of leaf bits, hypocotyls and roots were incubated on to the different callus Induction media, such as T_1 MS + 0.5 mg l^{-1} 2,4 - D + 0.3 mg l^{-1} kin +1g l^{-1} CH, T_2 MS + 0.5 mg l^{-1} 2,4 - D + 0.2 mg l^{-1} kin +1g l^{-1} CH, T_3 MS + 0.5 mg l^{-1} 2,4 - D + 0.1 mg l^{-1} kin + 1g l^{-1} CH, T_4 MS + 1.0 mg l^{-1} 2,4 – D + 0.3 mg l^{-1} kin +1 g l^{-1} CH, T_5 MS + 1.0 mg l^{-1} 2,4 - D + 0.2 mg l^{-1} kin + 1 g l^{-1} CH, T_6 MS + 1.0 mg l^{-1} 2,4 - D + 0.1 mg l^{-1} kin + 1 g l^{-1} CH, T_7 MS + 1.5 mg l^{-1} 2,4 - D + 0.3 mg l^{-1} kin + 1 g l^{-1} CH, T_8 MS + 1.5 mg l^{-1} 2,4 - D + 0.2 mg l^{-1} kin + 1 g l^{-1} CH, T_9 MS + 1.5 mg l^{-1} 2,4 - D + 0.1 mg l^{-1} kin + 1 g l^{-1} CH, T_{10} MS + 2.0 mg l-^{1} 2,4- D + 0.3 mg l^{-1} kin + 1 g l^{-1} CH, T_{11} MS + 2.0 mg l^{-1} 2,4 - D + 0.2 mg l^{-1} kin + 1g l^{-1} CH, T_{12} MS + 2.0 mg l^{-1} 2,4 - D + 0.1 mg l^{-1} kin + 1 g l^{-1} CH, T_{13} MS + 0.5 mg l^{-1} IAA + 0.3 mg l^{-1} kin + 1 g l^{-1} CH, T_{14} MS + 1.0 mg l^{-1} IAA + 0.3 mg l^{-1} kin + 1 g l^{-1} CH, T_{15} MS + 1.5 mg l^{-1} IAA + 0.3 mg l^{-1} kin + 1 g l^{-1} CH, T_{16} MS + 2.0 mg l^{-1} IAA + 0.3 mg l^{-1} kin + 1 g l^{-1} CH.

Statistical analysis: The observations recorded were statistically analyzed by subjecting the data to Factorial Completely Randomized Design, designed by (Gomez and Gomez, 1984). Level of significance was determined by using standard analysis of variance. Differences among mean values were assessed by LSD. The data obtained with per cent values were subjected to arc sine transformation.

RESULTS AND DISCUSSION

Effect of hormones on callus induction: Auxins were used to induce cell division and root differentiation in tissue culture medium. Among them, 2,4-D is widely used for callus induction. In the present study, MS medium containing 1.0 g l^{-1} casein hydrolysate was supplemented with various levels of 2,4-D *viz*., 0.5, 1.0, 1.5 and 2.0 mg l^{-1} and also IAA at 0.5, 1.0, 1.5 and 2.0 mg l^{-1} combination with cytokinin (kinetin). A differential influence of various concentrations of 2,4-D, IAA in callus behaviour was observed. Addition of adequate levels of synthetic auxins such as 2,4-D in to a basal medium resulted in prolific callus formation in maize tissue culture. Callus could not be induced in N_6 medium in the absence of 2,4-D (Binott *et al*., 2008). The influence of different concentration of 2,4-D, IAA alone or in combination with 0.3 mg l^{-1} kinetin were recorded maximum, callus induction percentage, number of days for callus induction and fresh weight of callus on 28 th day. This is in accordance with the findings of Ansari (1997) and Shohael *et al*. (2003) in maize. The complex action of kinetin was observed by Inoue and Maeda (1982) in rice. They explained the effect of kinetin on callus induction could be promotive or inhibitory depending upon the kind and concentration of auxin in the medium.

In the present study, MS medium containing 1.0 g l^{-1} Casein Hydrolysate has been added with different levels of kinetin *viz*., 0.1, 0.2 and 0.3 mg l^{-1} in combination with auxin 2,4-D and IAA. The result showed that among different concentrations tried, 0.3 mg l^{-1} kinetin recorded higher values (60.44%) of callus induction per cent when compared to other levels. Since kinetin is susceptible to interactions with 2,4-D, there was variation in response. But when 0.3 mg l^{-1} of kinetin was combined with 2,4-D 1.5 mg l^{-1} (T_7), IAA 1.5 mg l^{-1} (T_{15}) recorded the higher values when compared to either 2,4-D or IAA alone. Similar results reported by Shohael *et al*. (2003), embryogenic calli formation was high when N_6 medium supplemented with L-Proline 2.3 g l^{-1}, casein hydrolysate 200 mg l^{-1}, 2,4-D 1.0 mg l^{-1} and Kinetin 0.1 mg l^{-1}. Abebe *et al*. (2008) also reported, when 3 mg l^{-1} of 2,4-D was combined with 0.5 mg l^{-1} kinetin recorded highest callus induction per cent. Among the genotypes the maximum percentage of callus induction was recorded by CO H (M) 5 (59.61%) followed by UMI 285 (56.96%) and UMI 757 (55.25%). The maximum percentage of callus induction response was recorded by seed followed by immature embryo the explants leaf bits recorded the poorest response for callus induction. Among the 16 treatments studied, T7 showed the maximum of 60.44% followed by T15 (56.79%) and T16 (56.33%) (Table 1).

Among the G x E interactions, the maximum callus induction percentage was observed in G_4E_1 (99.50%) followed by G_5E_1 (99.20%) and G_1E_1 (98.46%) with the treatment of T_{15} (MS + 1.5 mg / l IAA + 0.3 mg /l K + 1 g/l (H). The treatment T_{12} showed poor performance in all the explants and in all the genotypes. Among the treatment combination G_4 E_1 T_{15} recorded the maximum value of (99.50%) (9.20%) and (98.46%) for the genotypes COH (M) 5, UMI 285 and UMI 757 respectively. The genotypes UMI 615 and CO 1 had a slightly lower value compared to the other genotypes.

Table 1. Callus induction per cent from different explants at various levels of hormones

T/ GE	T_1	T_2	T_3	T_4	T_5	T_5	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}	T_{13}	T_{14}	T_{15}	T_{16}	Mean
G_1E_1	90.74	88.34	82.76	86.52	84.58	85.47	96.12	92.18	91.56	90.64	57.09	78.15	86.46	92.57	98.46	95.38	**87.31**
G_1E_2	89.80	87.96	85.19	83.50	89.80	89.80	90.51	89.81	85.45	84.27	76.92	74.08	87.52	95.38	95.38	93.59	**87.43**
G_1E_3	51.59	65.27	59.58	55.48	54.62	63.81	61.03	54.61	51.86	59.25	49.08	49.11	64.70	60.17	65.59	64.18	**58.12**
G_1E_4	34.55	22.63	28.73	31.15	26.25	28.15	27.52	28.36	39.55	25.18	35.48	36.27	30.35	32.18	35.31	32.34	**30.87**
G_1E_5	10.84	13.65	11.32	12.01	13.32	12.76	18.34	13.18	13.57	10.82	11.25	10.17	10.18	10.82	14.38	13.97	**10.15**
Mean	**55.50**	**55.57**	**53.52**	**53.73**	**53.71**	**55.99**	**58.70**	**55.63**	**56.40**	**54.03**	**45.96**	**49.56**	**55.84**	**58.22**	**61.82**	**59.89**	**55.25**
G_2E_1	79.36	80.37	85.46	84.08	83.18	80.56	90.13	85.34	80.54	89.54	85.37	77.12	83.34	78.32	81.37	81.47	**82.84**
G_2E_2	76.45	78.71	84.08	81.46	87.05	86.14	92.60	90.73	85.15	87.05	78.69	76.14	82.76	75.14	78.70	75.14	**82.24**
G_2E_3	51.52	46.32	49.24	57.32	52.14	48.86	58.25	57.42	47.76	54.63	48.12	45.86	50.94	54.48	48.15	57.34	**51.77**
G_2E_4	19.47	18.42	17.25	22.56	28.59	23.79	31.15	27.58	30.47	24.47	19.59	13.23	20.57	21.74	18.60	28.21	**22.85**
G_2E_5	14.32	16.51	12.50	15.60	11.57	10.86	13.42	18.05	12.56	11.56	14.28	10.02	14.90	18.20	17.32	16.17	**14.24**
Mean	**48.22**	**48.06**	**49.71**	**52.20**	**52.51**	**50.04**	**57.11**	**55.82**	**51.30**	**53.45**	**49.21**	**44.47**	**50.50**	**49.58**	**48.83**	**51.67**	**50.79**
G_3E_1	82.76	81.84	87.02	85.14	82.14	85.18	90.58	87.97	90.08	92.60	82.21	80.37	85.14	90.74	92.58	91.27	**86.68**
G_3E_2	81.80	85.35	85.13	80.48	88.15	90.12	87.95	87.96	89.73	87.52	83.50	79.63	86.65	86.42	83.35	81.48	**85.32**
G_3E_3	59.57	65.28	58.25	60.18	53.28	52.15	53.71	54.62	55.47	50.93	53.6	50.84	59.90	53.35	48.27	54.30	**55.82**
G_3E_4	31.49	32.56	24.47	45.78	27.60	26.54	42.20	40.56	28.38	21.84	22.28	20.39	36.54	38.32	35.42	32.52	**31.68**
G_3E_5	12.92	12.42	12.56	17.32	10.58	14.25	16.24	14.38	11.08	16.28	11.15	10.91	17.38	11.56	13.56	12.29	**13.43**
Mean	**53.71**	**55.49**	**53.49**	**57.78**	**52.35**	**53.65**	**58.14**	**57.10**	**54.95**	**53.83**	**50.57**	**48.43**	**57.12**	**56.08**	**54.64**	**54.37**	**54.48**
G_4E_1	91.65	89.82	87.04	92.25	90.74	98.35	96.27	95.52	92.57	90.58	88.34	87.04	93.52	94.20	99.50	96.30	**92.73**
G_4E_2	89.80	87.97	85.17	93.27	9.78	92.70	93.55	93.84	90.58	80.18	87.03	82.41	91.75	90.72	98.13	97.32	**90.26**
G_4E_3	64.81	64.62	57.32	62.20	59.25	64.82	88.13	60.19	60.27	50.92	59.57	57.42	67.60	66.96	70.36	67.58	**63.87**
G_4E_4	30.16	28.54	40.39	30.57	32.44	45.24	65.61	32.78	28.76	38.14	27.61	28.13	26.15	30.18	37.78	35.52	**34.88**
G_4E_5	13.06	15.80	12.95	20.38	19.50	18.25	20.21	18.87	17.06	12.45	13.32	11.57	16.84	15.83	18.45	16.85	**16.33**
Mean	**57.90**	**57.35**	**56.57**	**59.73**	**58.34**	**63.87**	**72.75**	**60.24**	**57.85**	**54.45**	**55.17**	**53.31**	**59.17**	**59.58**	**64.84**	**62.71**	**59.61**
G_5E_1	90.30	85.37	87.32	90.02	94.32	92.57	93.38	92.14	92.51	87.62	85.42	83.32	81.97	95.36	99.20	96.28	**90.48**
G_5E_2	87.36	84.21	85.12	84.27	87.98	87.80	92.60	89.85	90.18	86.82	81.47	82.39	88.85	94.18	94.36	95.39	**88.36**
G_5E_3	65.74	55.70	60.27	55.87	60.17	60.14	70.15	59.24	57.40	59.27	59.56	53.71	65.74	65.26	68.52	65.74	**61.46**
G_5E_4	30.12	38.44	29.35	28.68	42.20	32.78	30.28	29.18	25.26	25.56	27.51	22.35	26.38	31.63	33.50	32.36	**30.34**
G_5E_5	15.84	15.20	10.76	12.05	17.94	17.56	12.62	14.15	17.57	15.48	11.23	11.10	13.17	12.87	14.87	14.87	**14.22**
Mean	**57.87**	**55.78**	**54.56**	**54.18**	**60.52**	**58.17**	**59.81**	**56.91**	**56.58**	**54.95**	**53.04**	**50.57**	**55.22**	**59.86**	**62.29**	**60.93**	**56.96**
G_6E_1	73.12	72.26	71.58	78.17	76.18	75.14	88.87	78.14	74.18	82.12	80.14	66.57	80.13	78.13	80.36	79.48	**77.16**
G_6E_2	75.41	73.54	70.47	80.15	80.05	79.44	91.13	80.53	78.76	76.54	78.43	58.48	79.56	72.56	76.58	73.39	**76.37**
G_6E_3	47.56	45.28	44.28	56.42	55.40	50.38	56.44	50.196	49.14	42.48	40.47	35.17	49.18	53.14	50.32	54.15	**48.75**
G_6E_4	18.46	17.48	16.57	25.14	24.15	22.87	30.17	25.16	20.86	18.54	17.58	16.18	19.56	20.16	22.12	25.10	**21.25**
G_6E_5	12.14	10.02	10.24	10.18	10.08	10.12	14.13	10.13	11.86	11.48	10.52	10.52	12.18	14.38	12.15	10.18	**11.26**
Mean	**45.34**	**43.72**	**42.63**	**43.95**	**49.17**	**47.59**	**56.15**	**48.83**	**46.46**	**46.23**	**44.83**	**37.38**	**48.12**	**47.67**	**48.31**	**48.46**	**46.58**
T(M)	**53.09**	**52.66**	**51.75**	**53.60**	**54.43**	**54.89**	**60.44**	**55.76**	**54.01**	**52.82**	**49.80**	**47.29**	**54.33**	**55.17**	**56.79**	**56.33**	

	SEd	**CD (0.05)**	**CD (0.01)**
G x Ex T interaction	0.82	1.60	2.11

Table 2. Number of days for callus induction from different explants at various levels of hormones.

T/GE	T_1	T_2	T_3	T_4	T_5	T_5	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}	T_{13}	T_{14}	T_{15}	T_{16}	Mean
G_1E_1	16.81	16.27	16.12	16.40	16.42	16.43	15.43	15.68	16.72	16.41	16.57	16.74	16.29	16.30	15.08	16.32	**16.25**
G_1E_2	17.34	18.21	16.89	17.34	17.19	17.56	16.41	17.12	17.32	16.80	17.18	17.75	17.10	17.42	16.14	17.45	**17.20**
G_1E_3	17.44	18.20	18.43	17.42	17.52	17.62	17.20	17.40	17.41	18.45	18.45	18.35	17.68	17.97	16.89	18.13	**17.91**
G_1E_4	18.42	18.56	19.10	18.41	18.36	18.42	18.21	18.98	18.35	19.14	19.54	19.34	18.10	17.98	17.91	18.10	**18.56**
G_1E_5	19.48	19.51	20.12	19.43	19.52	19.48	19.23	19.38	19.33	20.15	20.24	20.52	19.65	18.99	18.94	19.13	**19.57**
Mean	**17.89**	**18.15**	**18.13**	**17.80**	**17.80**	**17.90**	**17.29**	**17.71**	**17.83**	**18.19**	**18.39**	**18.54**	**17.76**	**16.53**	**16.99**	**17.83**	**17.90**
G_2E_1	16.45	17.42	17.54	17.21	16.53	16.88	16.12	16.32	17.22	16.45	17.53	18.02	16.34	16.44	15.56	16.53	**16.85**
G_2E_2	18.23	18.43	18.10	17.76	18.02	18.10	17.20	17.71	18.12	18.23	17.90	18.55	17.35	17.10	16.45	16.78	**17.75**
G_2E_3	18.53	18.03	18.33	18.10	18.38	18.31	17.56	18.20	18.11	18.53	18.57	18.42	18.41	18.24	17.17	17.70	**18.16**
G_2E_4	19.55	19.02	19.34	19.12	19.38	19.30	18.56	19.21	19.13	19.54	19.65	19.47	19.23	19.33	18.16	18.16	**19.13**
G_2E_5	16.78	20.58	16.02	20.62	15.32	20.62	19.57	20.35	20.70	15.80	20.60	20.55	20.25	20.31	19.15	19.70	**19.19**
Mean	**17.91**	**18.69**	**17.87**	**18.56**	**17.53**	**18.64**	**17.80**	**18.36**	**18.66**	**17.71**	**18.85**	**19.00**	**18.36**	**18.28**	**17.29**	**17.77**	**18.22**
G_3E_1	16.12	15.45	15.76	16.35	16.34	15.58	15.35	16.01	15.58	15.79	16.13	16.78	16.02	16.54	16.10	15.90	**15.98**
G_3E_2	16.56	17.00	17.36	16.76	16.75	17.20	16.42	16.23	17.25	17.45	16.42	17.43	17.13	16.42	16.57	16.18	**16.82**
G_3E_3	18.14	18.36	18.72	17.45	17.48	17.58	17.41	17.50	17.62	18.15	18.52	18.23	17.14	17.13	17.02	17.21	**17.72**
G_3E_4	1922	19.35	19.23	18.43	18.45	18.62	18.42	18.38	18.54	19.15	17.50	19.23	18.12	18.15	18.01	18.23	**18.56**
G_3E_5	20.25	20.26	20.15	19.44	19.48	19.51	19.24	19.16	19.65	20.43	18.48	20.24	19.15	19.11	19.00	19.24	**19.55**
Mean	**18.06**	**18.08**	**18.24**	**17.69**	**17.71**	**17.69**	**17.37**	**17.46**	**17.73**	**18.19**	**17.41**	**18.38**	**17.51**	**17.47**	**17.34**	**17.35**	**17.73**
G_4E_1	14.98	16.40	16.49	16.32	16.13	15.28	16.31	15.25	16.45	15.20	16.31	16.42	17.03	16.51	15.10	15.21	**15.96**
G_4E_2	15.11	17.46	17.34	17.15	17.52	17.24	16.52	17.23	17.02	18.03	17.58	16.41	16.90	16.34	16.00	16.03	**16.86**
G_4E_3	16.03	17.94	18.57	17.82	17.68	18.35	17.15	18.15	18.45	18.20	18.76	17.43	17.23	17.52	16.54	16.83	**17.67**
G_4E_4	17.11	19.02	19.14	18.26	18.92	19.23	18.10	19.10	19.32	19.01	19.05	20.48	18.25	18.36	17.03	17.32	**18.60**
G_4E_5	17.12	20.25	19.52	19.54	19.76	20.25	19.12	19.12	19.45	20.05	19.36	19.72	19.13	19.51	18.52	18.45	**19.30**
Mean	**16.07**	**18.21**	**18.21**	**17.82**	**18.00**	**18.07**	**17.44**	**17.78**	**18.14**	**18.09**	**18.21**	**18.09**	**17.71**	**17.65**	**16.64**	**16.77**	**17.68**
G_5E_1	16.54	17.21	17.15	16.25	16.86	16.56	16.32	16.61	16.95	16.61	17.57	17.87	16.51	16.86	16.20	16.84	**16.80**
G_5E_2	17.45	18.43	17.56	18.13	16.88	17.02	16.52	16.82	17.57	18.20	18.42	18.53	18.14	16.54	16.43	16.56	**17.45**
G_5E_3	18.14	18.21	18.57	17.45	18.15	18.21	17.52	18.10	18.45	18.13	18.53	18.55	17.46	17.35	17.12	17.38	**17.96**
G_5E_4	19.13	19.20	19.55	18.48	18.24	19.22	18.50	18.24	18.87	19.41	19.57	19.56	18.45	18.31	19.10	18.40	**18.88**
G_5E_5	20.15	19.23	20.54	19.85	19.16	20.24	19.56	19.18	19.48	20.42	20.66	20.55	19.42	19.56	19.05	19.38	**19.77**
Mean	**18.28**	**18.46**	**18.67**	**18.03**	**17.86**	**18.25**	**17.68**	**17.79**	**18.26**	**18.55**	**18.95**	**19.01**	**17.99**	**17.72**	**17.58**	**17.71**	**18.17**
G_6E_1	16.46	17.43	17.53	17.23	16.54	16.90	16.13	16.33	17.24	16.47	17.54	18.03	16.35	16.50	15.58	16.54	**16.80**
G_6E_2	18.25	18.45	18.14	17.76	18.05	18.11	17.21	17.74	18.14	18.25	17.91	18.54	17.36	17.12	16.47	16.80	**17.76**
G_6E_3	18.55	18.04	18.35	18.13	18.39	18.30	17.57	18.20	18.10	18.54	18.58	18.45	18.43	18.25	17.41	17.74	**18.19**
G_6E_4	19.57	19.05	19.35	19.15	19.40	19.32	18.56	19.25	19.14	19.58	19.67	19.48	19.25	19.34	18.18	18.18	**17.94**
G_6E_5	16.80	20.60	16.03	20.65	15.35	20.63	19.58	20.37	20.80	15.84	20.62	20.56	20.47	20.34	19.17	19.72	**19.22**
Mean	**17.93**	**18.71**	**17.88**	**18.58**	**17.55**	**18.65**	**17.81**	**18.38**	**18.68**	**17.74**	**18.86**	**19.01**	**18.37**	**18.31**	**17.36**	**17.79**	**17.98**
T(M)	**17.69**	**18.38**	**18.17**	**18.08**	**17.74**	**18.20**	**17.56**	**17.91**	**18.22**	**18.08**	**18.45**	**18.67**	**17.95**	**17.66**	**17.20**	**17.54**	
		SEd		**CD (0.05)**		**CD (0.01)**											
G x Ex T interaction		0.71		1.39		1.83											

Table 3. Fresh weight (mg) of callus from different explants at various levels of hormones on 28th day

T/GE	T_1	T_2	T_3	T_4	T_5	T_5	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}	T_{13}	T_{14}	T_{15}	T_{16}	Mean
G_1E_1	3428	3406	3465	3420	3428	3426	3450	3422	3415	3420	3405	3415	3436	3612	3534	3510	**3436.93**
G_1E_2	3220	3205	3203	3248	3260	3298	3328	3245	3273	3276	3248	3255	3237	3258	3460	3392	**3275.38**
G_1E_3	1043	1021	976	1023	1035	1001	1015	1043	1008	991	1008	988	1034	1043	1038	1041	**1019.26**
G_1E_4	597	613	583	610	591	585	601	627	590	575	572	571	614	603	641	607	**598.75**
G_1E_5	425	417	320	415	394	328	394	428	401	371	388	365	419	422	427	415	**395.56**
Mean	**1742.6**	**1732.40**	**1709.40**	**1743.20**	**1741.60**	**1727.40**	**1757.60**	**1753**	**1737.4**	**1726.60**	**1724.20**	**1718.8**	**1748**	**1747.60**	**1820**	**1793**	**1745.20**
G_2E_1	3423	3400	3456	3413	3421	3420	3440	3403	3410	3411	3400	3410	3425	3388	3528	3501	**3428.06**
G_2E_2	3215	3195	3197	3240	3254	3294	3321	3224	3256	3268	3233	3225	3230	3241	3454	3324	**3260.68**
G_2E_3	1038	1013	970	1028	1027	993	998	1038	995	980	995	981	1021	1037	1027	1032	**1010.81**
G_2E_4	593	611	574	601	583	577	593	621	583	558	566	564	588	589	625	600	**589.13**
G_2E_5	420	413	315	411	384	320	390	417	390	352	381	355	403	417	421	411	**387.50**
Mean	**1737.8**	**1726.4**	**1702.4**	**1738.6**	**1733.8**	**1720.8**	**1748.4**	**1740.6**	**1726.8**	**1713.8**	**1715**	**1707**	**1733.4**	**1734.4**	**1811**	**1773.6**	**1735.2**
G_3E_1	3425	3400	3461	3415	3425	3423	3447	3421	3413	3416	3401	3413	3432	3410	3531	3507	**3433.75**
G_3E_2	3218	3201	3200	3245	3257	3295	3323	3242	3270	3270	3243	3254	3235	3247	3456	3326	**3267.63**
G_3E_3	1041	1018	973	1021	1031	997	1011	1041	1004	987	1004	985	1031	1041	1032	1035	**1015.75**
G_3E_4	595	612	580	603	587	581	598	625	588	573	570	567	611	602	638	603	**595.81**
G_3E_5	422	415	318	413	390	323	393	423	397	368	386	364	417	420	425	414	**393.06**
Mean	**1740.2**	**1729.2**	**1706.6**	**1739.4**	**1738**	**1723.8**	**1754.4**	**1750.4**	**1734.4**	**1722.8**	**1720.8**	**1716.6**	**1745.2**	**1744**	**18165.4**	**1777**	**1741.20**
G_4E_1	3435	3411	3476	3425	3432	3431	3473	3425	3418	3423	3408	3418	3440	3423	3538	3514	**3443.13**
G_4E_2	3525	3216	3298	3263	3267	3302	3329	3252	3288	3280	3258	3260	3245	3276	3465	3398	**3288.87**
G_4E_3	1051	1030	989	1027	1044	1009	1015	1048	1013	998	1015	993	1041	1048	1054	1045	**1026.25**
G_4E_4	601	612	588	615	593	592	605	625	595	581	576	580	615	618	645	610	**603.18**
G_4E_5	432	430	319	420	395	393	398	427	408	378	392	371	420	427	432	425	**404.18**
Mean	**1748.8**	**1739.8**	**1734**	**1750**	**1746.2**	**1745.4**	**1764**	**1755.4**	**1744.4**	**1732**	**1729.8**	**1724.4**	**1752.2**	**1758.4**	**1826.8**	**1798.4**	**1753.13**
G_5E_1	3430	3408	3470	3423	3430	3428	3471	3421	3416	3421	3407	3416	3438	3420	3536	3513	**3441.06**
G_5E_2	3224	3207	3282	3258	3263	3300	3325	3250	3280	3278	3252	3257	3240	3271	3464	3395	**3440.75**
G_5E_3	1048	1026	980	1025	14042	1004	1013	1046	1012	995	1013	990	1039	1045	1050	1042	**1023.68**
G_5E_4	600	615	586	612	595	587	602	620	593	580	575	574	613	611	643	608	**600.87**
G_5E_5	428	420	320	418	396	391	395	425	404	373	390	368	420	423	431	421	**401.43**
Mean	**1746**	**1735.20**	**1727.60**	**1747.2**	**1745.2**	**1742**	**1761.2**	**1752.4**	**1741**	**1729.4**	**1727.4**	**1721**	**1750**	**1754**	**1824.8**	**1795.8**	**1750**
G_6E_1	2902	2890	3115	3115	3120	3128	3322	3301	3308	3308	3200	3310	3318	3187	3524	3498	**3221.43**
G_6E_2	2721	2752	2874	2895	3215	3007	3117	3103	3248	3148	3118	3107	3005	3105	3328	3118	**3053.75**
G_6E_3	787	810	958	1001	1021	896	990	1005	991	888	887	890	1004	1018	1017	1001	**947.75**
G_6E_4	520	585	571	565	581	570	590	528	575	547	556	554	575	575	624	587	**536.43**
G_6E_5	359	411	310	398	376	318	378	430	280	358	375	348	401	414	428	420	**375.25**
Mean	**1457.8**	**1489.6**	**1565.6**	**1661.6**	**1662.6**	**1583.8**	**1679.4**	**1673.4**	**1680.4**	**1649.6**	**1627.2**	**1641.8**	**1660.6**	**1659.2**	**1784.2**	**1724.8**	**1637.6**
T(M)	**1695.5**	**1692.1**	**1690.9**	**1730**	**1727.9**	**1699.7**	**1744.2**	**1737.5**	**1727.4**	**1712.4**	**1707.4**	**1704.9**	**1731.6**	**1732.9**	**1813.9**	**1777.1**	
		SEd		**CD (0.05)**		**CD (0.01)**											
G x Ex T interaction		16.57		32.53		42.79											

Effect of explant: In the present study, investigations were made using five explants seed (E1), immature embryo (E2), leaf bits (E3), root (E4) and hypocotyls (E5). These explants were cultured with various levels of hormones and organic supplements and variation in their capacity in callus response was studied.

The studies indicated that maximum percentage of callus induction response was observed in seed followed by immature embryo and leaf bit recorded the poorest response of callus induction. Similar results were already reported by Shohael *et al*. (2003) in maize. The high callus responding nature and culturability of mature seed was in accordance with the reports of Delporte *et al.* (2001) in wheat and Bijy (2002) in rice. Al-Abed *et al*. (2006) and Sikandar *et al.* (2007) reported that the mature seed scutellum is the best explant for high totipotent embryogenic callus initiation and they also reported that the plant regeneration from coleoptile and root segments was unsuccessful. The high culturability of immature embryo was in agreement with the reports of Oduor *et al.* (2006). Benson, (2000) and Huang and Wei (2004) reported the juvenile tissues are usually more responsive to tissue culture than mature ones.

In the present study, studies regarding explants performance immature embryo as well as seed was more (or) less similar and best. Hypocotyl, root and leaf bits were lagging behind and the poor response of leafbits was also reported by Vinothini (2004) in rice. Chand and Sahrawat (2000) reported high callus induction and plant regeneration using root explants of barley. In contrast, Khaleda and Al-Forkan (2006) reported that root explant showed poor response be due to the fact that the calli derived from those explants were not totipotent for plant regeneration in rice.

Number of days for callus induction (days): In callusing duration studies, for early callus induction inferred that the use of 1.5 mg l^{-1} IAA in combination with 0.3 mg l^{-1} kinetin (T_{15}) followed by 2.0 mg l^{-1} IAA + 0.3 mg l^{-1} kinetin (T_{16}) and 1.5 mg l^{-1} 2,4-D + 0.3 mg l^{-1} kinetin (T_7) was the best. Among the explants, seed recorded the earlier induction of callus followed by immature embryo. The leaf bit recorded maximum days for callus induction.

Among the genotypes the earlier induction of callus was observed in CO H (M) 5 (G_4) (17.68 days) followed by UMI 285 (G_5) 17.73 days. CO 1 (G_6) recorded the maximum days for callus induction (18.22). The influence of genotypes on callus induction in maize was reported already by Green and Philips, (1975) and Tomes and Smith, (1985). Bronsema *et al.* (1997) indicated that the genetic information needed for embryogenic callus formation of A 632 as female parent, was transferred through A 188 pollen. The results revealed that among the explants, seed (E_1) recorded the earlier induction of callus (16.22 day) followed by immature embryo (E_{-2}). The leaf bit explant took maximum days for callus induction (19.27 days). Among the G x E interactions, G_1E_1, G_4E_1, G_2E_1 recorded the earlier days with T_{15} (MS + 1.5 mg / l IAA + 0.3 mg /l K + 30 g / l maltose) 15.08 days, 15.10 days and 15.56 days respectively. The interactions G_6E_5 with T_9 recorded longer days for callus induction (20.80 days). The results on number of days for callus induction for the hormonal combination revealed significant 1.39 differences among the treatments. MS + 1.5 mg / l IAA + 0.3 mg/ l K + 30 g/l maltose (T_{15}) treatment recorded the minimum number of days for callus induction 17.20 days followed by T--$_{12}$ (18.67 days) (Table 2).

Under various levels of organic supplements, significantly superior callus induction per cent on immature embryo was noticed in CO H (M) 5, UMI 285 and UMI 757 when compared to other types. Study in seed emphasized the capacity of CO H (M) 5 and UMI 285 with higher per cent values and indicated its superiority over other genotypes. Similarly CO H (M) 5 and UMI 285 genotypes showed significant values in the case of root and hypocotyl, where as other genotypes stood inferior. When study was conducted on duration of callusing, minimum number of days was observed for immature embryo in CO H (M) 5, UMI 285 and UMI 757 when compared to other genotypes. Seed culture confirmed again minimum duration induction for COH (M) 5 followed by UMI 285 and UMI 112 and they stood superior to other genotypes. In the case of root and hypocotyl, significantly higher values were noticed in COH (M) 5 followed by UMI 112 as compared to others.

In all the above studies, COH (M) 5 was performing extremely well under all treatments. This can be attributed to its already high responding nature and it is a tissue culture friendly type. UMI 285, UMI 757, UMI 112, UMI 615 and CO 1 also high response, where as CO 1 lagged behind all treatments. Such type of varietal variation to culture response was reported by other researchers Hodges *et al.* (1986) in maize, Bronsema *et al.* (1997) in maize Agarwal *et al.* (2006) in rice and Binott *et al.* (2008) in maize.

Fresh weight of callus on 28th day (mg): Callus fresh weight on 28 th day, significantly superior at T_{15} (1.5 mg l^{-1} IAA) followed by T_{16} (2.0 mg l^{-1} IAA) and T_7 (1.5 mg l^{-1} 2,4-D) (Table 20). Among the five explants, seed recorded maximum fresh weight of callus followed by immature embryo. This was in agreement with the report of Ansari, (1997), he cultured on 2,4-D ranged from 1.5 mg l^{-1} to 2.0 mg l^{-1} with different media *viz*., (MS) Murashige and Skoog, (N_6) Chu, (B_5) Gamborg B5 Medium , (YP) Yeast Media and (LS) Linsmaier and Skoog Medium. Callus induction was high in (MS) Murashige and Skoog and (N_6) Chu medium when compared to other media, which was due to the differences on major nutrients present in these basal media.

Among the genotypes, the maximum callus weight was

observed in COH (M) 5 (G_4) 1753.13 mg followed by UMI 285 (G_5) (1750 mg) and UMI 757 (G_1) (1745.2 mg). The genotype CO 1 (G_6) recorded the minimum callus weight of 1444.62 mg. The results revealed that among the explants, the seed explant (E_{-1}) recorded the maximum weight of 3400.73 mg followed by immature embryo (E_2) 3264.51 mg. The leaf bit explant (E_{-5}) had minimum fresh weight 392.83 mg. The results of fresh weight on 28th day of the hormonal combination revealed significant differences among the treatments. The treatments T_{15} (MS + 1.5 mg /l IAA + 0.3 mg / l K 30 g/l maltose), T_{16}- (MS + 2.0 mg /l IAA + 0.3 mg / l K + 30 gl/ maltose) and T_7 (MS + 1.5 mg / l 2,4 D + 0.3 mg / l K + 30 g/l maltose) recorded the maximum weight of 1813.9 mg, 1777.1 mg and 1744.2 mg respectively. The treatment T_3 recorded the minimum weight of callus (1690.90 mg). Among the G x E interactions G_4 E_1 recorded the maximum weight of 3443.13 mg, followed by G_5E_1 (3441.06 mg) and G_5E_2 (3440.75 mg). The treatment combination G_6 E_5, G_2E_5, G_3E_5 recorded the minimum weight of 375.25 mg, 387.50 mg and 3930.06 mg respectively (Table 3).

Among the G x E x T interactions G_4E_1 T_{15} recorded the maximum weight of callus (3538 mg) followed by $G_5E_1T_{15}$ (3536 mg). The treatments $G_6E_5T_9$, $G_6E_5T_3$ and $G_2E_5T_3$ recorded the minimum of callus of 280 mg, 310 mg and 315 mg respectively. Among the G x E x T interactions $G_1E_1T_{15}$ recorded the minimum value of 15.02 days followed by $G_{-4}E_1T_{15}$ (15.10 days), while the treatment combination $G_6E_5T_9$ recorded the maximum number of days for callus induction (20.80 days).

Conclusion

Transformation of maize plants was made successfully by standardization of tissue culture experiments. The study concluded that 0.3 mg/l of kinetin combined with 1.5 mg/l 2,4-D and 1.5 mg/l IAA has recorded highest callus induction percentage. The genotypes COH(M) 5 with Immature embryo as a explant exhibited earlier callus induction and maximum callus weight. These optimized tissue culture protocol, will be used for transformation of resistance to biotic and abiotic stress in maize in future.

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