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EFFECT OF COB PIECE SIZE, KERNEL POSITION AND MS SALT, SUCROSE CONCENTRATION DURING *IN VITRO* KERNEL DEVELOPMENT OF MAIZE *(ZEA MAYS L.)*

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In vitro kernel growth was studied in maize (*Zea mays* L.) hybrid Ganga-2 starting at 7 days after pollination for 8 days. Six kernels per cob piece (with kernel to cob ratio of 1:6 on weight basis) gave kernel growth @ 4.55 mg kernel^{-1} day^{-1}, which was similar to 4.60 mg kernel^{-1} day^{-1} observed in the mid ear kernel at 15 days after pollination under field conditions. The kernels at sub-middle ear head gained maximum weight in a period of 8 days as compared with kernels of sub-apical and sub-bottom ear head. 10% M.S. salt strength and 30 g/l sucrose were sufficient to gave good growth and that further increase in both M.S. salt and sucrose concentration had no effect on growth rate. The *in vitro* kernel growth technique provides a tool to investigate biotic and abiotic factors governing the maize yield.

Keywords : Cob; Dry matter; *In vitro*; Sucrose; *Zea mays* L.

Introduction

The tropical Indian maize has high harvest index however grain yield is far less than the temperate maize. Various yield determinant constraints have been indentified through field and loboratory studies. The most important of them is the poor transport and partitioning of total dry matter to the grain[1]. Maize grain development following *in vitro* kernel culture starting at 4 to 5 days after pollination (DAP) has been used to study the effect of genotype[2], temperature[3], amino acid uptake and incorporation into Zeins[4], sugar utilization[5] and the interaction of carbon and nitrogen supplied on kernal development[6]. We examined effect of cob piece amount, kernel position along the ear and variable MS salt strength and sucrose concentration on growth and development of *in vitro* culture of maize kernel.

Materials and Methods

Maize (variety Ganga-2) plants were raised at instructional farm, Rajasthan college of Agriculture, Udaipur, during kharif season of 1999, using recommended agronomic practices. Ear (pistillate inflorescence) of the maize plant were covered with bags before silk (style) emergence. After silk emergence, the ears were hand pollinated with collected pollen. The ears of seven days after pollination (7 DAP) were used for *in vitro* kernel culture.

Effect of cob piece amount on growth : Cob blocks were excised from ear middle part and sliced horizontally, 2-3 mm below the point of glume attachment without disrupting the structural integrity of the cob piece. The white central pith removed and cut into 6 (3x2 paired rows), 9 (3x3 paired rows), 12 (4x3 paired rows) and 15 (5x3 paired rows) kernels per cob piece. The constant cob piece weight by 6 times the total of 6 kernels (i.e. about 2.47±0.1 g) or indicated for each experiment separately was taken for each treatment. The kernel cob ratio by weight for 6, 9, 12 and 15 kernels/ cob piece was 1x6, 1x4, 1x3 and 1x2.4, respectively. In one of the treatment, 6 kernels per cob block with five kernels removed without reducing the amount of cob piece was used[7].

Effect of kernel position along the earon growth : 7DAP cob was divided into three zones by slicing transversely into basal, middle and apical parts along the ear head positions respectiveley. Each zone was further subdivided into three segment and each middle sub part was used for culture. The cob blocks bearing 6 kernels (i.e. 1x6

kernel : cob ratio by weight) from respective zones were incubated.

Effect of MS salts strength and sucrose concentration on growth : Availability of sugar and salts supply can affect growth and development of culture kernels. This experiment was performed to assess the effect of variable medium salt strength and sucrose amount on kernel growth rate.

The cob blocks of 6 kernels were used as described in experiment-1, treatment number-2. The mean kernel growth rate was obtained on 12 media formulations in which sucrose and MS basal salts were varied (Table-3).

Cultural Condition : MS basal medium[8] PT-11 Fortified with 440 mg $CaCl_2$ and 500 mg activated charcoal was used for culture and standard conditions were followed. The cob pieces were seated into the medium. All the cob pieces were positioned upright as in the ear position. The flasks were incubated at room temperature (28±4°C) in the dark for 8 days.

Kernal fresh and dry weight : Intact kernels were excised by pressing forceps below the glume and weighed. Cob piece were weighed along with kernel before explanting due to the need to maintain integrity of cob and ovules tissue and because ovules (kernels) could not be weight seperately. However, initial values for kernel and cob tissues weight for respective samples obtained from corresponding positions on ear. The fresh and dry weight of the kernel before and after 8 days culture was recorded. After separating, kernels were dried at 100°C for 1 h and then to a constant weight at 60°C[9]. Kernel water content was calculated by substracting the dry weight from the fresh weight. The kernel growth rate i.e. mg/ kernel/day was calculated by dividing total number of days in culture.

Statistical analysis : "F" test[10] was applied to evaluate significance of data.

Results and Discussion

Effect of cob piece size on kernel growth and development : The growth of 7 DAP kernels at various kernel to cob ratio (both number and weight basis) for a period of 8 days during *in vitro* culture are presented (Table 1). Results reveal that 1:6 kernel ratio on weight basis gave the best growth showing a 2.0 and 3.3 fold increase in fresh and dry weight over explant. The growth rate decreased with increase in number of kernels (9, 12 and 15 kernels). All the kernels set on in lower cob ratio for 9, 12 and 15 kernels treatments. Developing kernels did not show any sign of deterioration. The best growth observed was similar to single kernel bearing cob piece with kernel: cob ratio of 1:6[7]. The attainment of kernel growth rate of 4.5 mg $kernel^{-1}$ day^{-1} from six kernels culture technique was similar to 4.60 mg $kernel^{-1}$ day^{-1} in the field developed mid ear kernel at 15 DAP confirmed the successful accomplishment of the *in vitro* kernel culture technique.

The present result indicate that the bearing of the kernels per cob piece had influence on lag phase duration. Which suggest a physiological interdependence of kernel : cob ratio on the kernel development processes. Therefore, solute supplied from the medium through the conducting phloem tissue of cob piece feeding the developing kernels is a possible cause for the interdependence. The observed results are consistent with the previous studies showed that amount of phloem tissue of the cob piece *in vitro* has effect on kernel development, water intake capacity, duration of lag phase, grain filling rate and duration of kernel growth[7,11,12].

During these 8 days period the cob piece gained 110±5 mg weight on dry weight basis (Data not shown in table). An increase of 20 mg cob dry weight from 7 to 14 days single ovule culture consisting 1:6 ovule:cob ratio have also been reported[13]. Apparently the cob piece-was not fully

Table 1. Effect of cob piece mass on growth of *in vitro* culture maize kernels.

No. of Kernels (Kernel : Cob)	Weight gain (mg kernel^{-1})		Water content (mg kernel^{-1})	Growth rate (mg kernel^{-1}day^{-1})		Water uptake (mg kernel^{-1} day^{-1})
	FB	DB		FB	DB	
1 Kernel (1:6) a	73.33	33.50	39.83	9.16	4.18	4.98
6 Kernel (1:6) b	71.94	33.33	38.61	8.99	4.16	4.82
9 Kernel (1:4) b	57.40	25.50	31.72	7.17	3.18	3.97
12 Kernel (1:3) b	36.52	17.33	19.19	4.56	2.16	2.40
15 Kernel (1:2.4)b	26.00	13.50	12.50	3.25	1.68	1.56
SEm ±	1.144	0.506	1.33	0.099	0.063	0.165
C.D. at 5%	3.60	1.60	4.19	3.12	0.20	0.520

- Kernels were cultured for 8 days on M.S. medium in triplicate, FB = fresh weight basis, DB = dry weight basis.
- The mean of initial kernel fresh/dry mass and moisture content at 7 DAP was 68.5±1.67 mg/10.02±0.35 mg and 58.28±1.5 mg respectively.
- a, indicate cob block bearing 6 kernels were excised and all kernels except one were removed (Felker, 1992) viz., cob : kernel ratio of 1:6 by size.
- b, indicate equal cob block weight of 2.47±0.1 g for 6, 9, 12 and 15 kernels, giving cob : kernel ratio of 1:6, 1:4, 1:3 and 1:2.4 by weight, respectively.

Table 2. Effect of kernel position along the ear on growth of *in vitro* culture maize kernels.

Kernels position along ear head	Weight gain (mg kernel^{-1})		Water content (mg kernel^{-1})	Growth rate (mg kernel^{-1}day^{-1})		Water uptake (mg kernel^{-1} day^{-1})
	FB	DB		FB	DB	
Sub-apical	35.00	15.00	20.00	4.37	1.87	2.50
Sub-middle	70.00	33.36	36.68	8.74	4.16	4.58
Sub-bottom	62.22	32.00	30.22	7.77	4.00	3.78
SEm ±	1.087	0.434	1.277	0.135	0.056	0.391
C.D. at 5%	3.76	1.50	4.421	0.673	0.274	1.353

- The Kernels were cultured on M.S. medium for 8 days in triplicate, FB = fresh weight basis, DB = dry weight basis.
- The mean of initial (i) sub-apical, (ii) middle and (iii) bottom kernel fresh/dry mass and water content at 7 DAP was (i) 45.55±0.82 mg /5.0±0.35 mg and 40.55±1.06 mg; (ii) 65.27±1.63 mg /9.5±0.15 mg and 55.77±1.72 mg; (iii) 62.21±0.91 mg / 9.0±0.11 mg and 53.21±0.85 mg, respectively.
- 6 Kernels cob piece^{-1} weighing 6 times the weight of 6 kernels was used for experiment.

Table 3. Effect of MS salt strength and sucrose concentration on growth of *in vitro* culture maize kernels.

Treatments	Weight gain (mg kernel^{-1})		Water content (mg kernel^{-1})	Growth rate (mg kernel^{-1}day^{-1})		Water uptake (mg kernel^{-1} day^{-1})
	FB	DB		FB	DB	
MS Salt Strength						
10%	80.64	36.28	44.35	10.08	4.54	5.54
25%	82.95	36.36	46.60	10.36	4.55	5.82
50%	69.62	34.33	35.28	8.70	4.29	4.41
100%	68.01	33.70	34.31	8.50	4.21	4.29
SEm ±	0.854	0.468	1.104	0.106	0.059	0.143
C.D. at 5%	2.495	1.365	3.223	0.310	0.172	0.417
Sucrose (gl^{-1})						
30	75.06	35.28	39.77	9.38	4.41	4.97
100	74.78	35.02	39.75	9.35	4.37	4.96
150	76.08	35.20	40.88	9.51	4.40	5.11
SEm ±	0.740	0.405	0.956	0.092	0.026	0.123
C.D. at 5%	NS	NS	NS	NS	NS	NS

- The Kernels were cultured on M.S. medium for 8 days in triplicate, FB = fresh weight basis, DB = dry weight basis.
- The mean of initial kernel fresh/dry mass and moisture content at 7 DAP was 68.18±1.0 mg/10.30±0.3 mg and 57.88±0.95 mg respectively.
- Kernel : Cob ratio of 1:6

developed when excised, since growth continued for 8 days in culture.

Position effect on kernel growth and development: Data in Table 2 show kernels development on *in vitro* culture with kernel : cob ratio of 1:6 bearing 6 kernels from subapical, sub-middle and sub-bottom ear position. Apical kernel accumulated dry matter 54.% more slowely than kernel at other section of the ear and had a 40.0% lower water content at 15 DAP. Apical ear section had about 30.0% lower cob weight than at mid-ear and bottom position of ear.

Kernels of sub-middle ear accumulated maximum fres and dry mass, which was twice than that of apical ear. The proximity of the mid-ear and bottom kernels due to acropetal pattern on the ear position resulted into more or less similar rate of dry matter accumulation, growth rate and kernel water uptake during 8 days in culture.

The observed developmental patterns at the three ear positions is consistent with the theory that the amount of conducting phloem tissue of the cob at the tip of the ear feeding the apical kernel

development or associated with higher number of fertilized kernels with limited assimilatate supply[11]. It is pertinent to note that the culture apical kernel growth parameter resembles in all respect to lower cob ratio bearing 12 kernels per cob piece (Table 1).

Effect of salt and sucrose concentration : The effect of four MS salt strength (10, 25, 50 and 100%) with medium containing 30, 100 and 150gl^{-1} sucrose on kernel growth parameter during 8 days *in vitro* cultures are showed in Table3. Maize kernel cultured with 10% salts (4.3 gl^{-1}) strength of the medium had 15.8 and 6.8% higher fresh and dry weight, 21.5% more water content over the average of 50 and 100% of medium salt strength. The experiment indicates that kernel growth seems most synchronize with lower salts than higher salts strength in the medium.

The growth and development of kernel under three sucrose level (i.e. 30, 100 and 150 gl^{-1}) were similar (Table 3), whereas, increasing sucrose concentration between 80 and 160 gl^{-1} in the medium enhanced kernel dry weight but no significant increase observed in kernel dry weight with medium containing more that 80 gl^{-1} sucrose[5]. Differences among these two results and this result may be attributable to genetic differences in plant material. These results indicated that sucrose amount seems to influence *in vitro* stimulated growth of vascular cob tissues rather than kernel development, which was higher in the current experiment i.e. 22.0 40.6 and 52.7% with 30, 100 and 150 gl^{-1} of sucrose, respectively (data not shown). This is consistent with the observed result[13]. Furthermore, the higher increase in cob growth indicated that sucrose absorption by the endosperm may be slower than the rate of accumulation in the cob pedicel and thus potentially could be limiting kernel development. This suggest that the developmental programming seems to occur prior to 7 DAP, when the explant was removed from the field plant. The genes contributed from the pollens (xenia effects) has hastened the growth of vascular cob tissue rather than kernel dry matter in response to sucrose concentration.

These results suggests that in any kernel, ear association studies cluster of kernels can be accommodated per cob piece by taking appropriate amount of cob tissue proportional to weight of kernels present on the cob block and salt sucrose concentration.

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