Micro propagation and field evaluation of strawberry in Bangladesh

M.K. Biswas[1*], R. Islam[2] and M. Hossain[2]

[1]National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan, Hubei, China, 430070
[2]Department of Botany University of Rajshahi, Bangladesh

Biswas, M.K., Islam, R. and Hossain, M. (2008). Micro propagation and field evaluation of strawberry in Bangladesh. Journal of Agricultural Technology 4(1): 167-182.

The *in vitro* multiple shoot regeneration was tried from runner tip explants of three strawberry clones. Different concentrations and combinations of BA, Kin and NAA were used in MS media for the above purpose. Best response towards multiple shoot regeneration was obtained on MS medium having 0.5 mg/l BA. Among the tested clones no significant differences were found shoot number and harvestable shoots. To enhance shoot multiplication low concentration of BA was recommended. Mean were significantly different for the traits NL, NC, NF and AFW among the tissue culture and runner derived plants. Analysis of variance showed that the strawberry clones under study were highly significant for most of the traits except PH and CZ. Our results reveal that phenotypic variance and phenotypic coefficient of variance were higher than the corresponding genotypic variance, environmental variance, genotypic coefficient of variance and environmental coefficient of variance.

Key words: Micropropagation, Strawberry, genetic parameters, path coefficient

Introduction

Strawberry is a perennial, stoloniferous herb belongs to the family *Rosaceae*, genus *Fragaria* and most widely consumed fruits throughout the world. It is one of the most popular fruits growing in the Northern hemisphere in temperate and sub temperate environment. There are many strawberry genotypes grown in tropical and subtropical environment but fruits of which are mostly unpalatable.

Strawberry is traditionally propagated vegetatively by rooted runners. To improve the strawberry varieties this method was not suitable due to incidence of many diseases infection and environmental hazards and resulting in the gradual degeneration of cultivars performance. The rate of strawberry propagation through conventional techniques is quite low and is difficult to maintain plant materials during summer in Bangladesh. Moreover, the conventional way of production is not adequate to meet the commercial demand. Several improvements of the technology have been

*Corresponding author: Manosh Kumar Biswas; e-mail: manosh24@yahoo.com

proposed by authors working with strawberry (Damiano, 1980; Drew *et al.,* 1986; Swartz, 1987) but the highest genotypic, physiological and morphological quality of micropropagated plants is produced by the method described by Boxus and co workers (Boxus, 1974; Jemmali *et al.,* 1995). Micro propagated strawberry plant has been introduced to prevent most of the plant and soil transmissible diseases.

In order to increase yield potential, information about genetic variability is necessary. The degree of success of genetic study and progress in the breeding work depends upon the magnitude of genetic variability in the available materials. To get idea of the genetic variability existing among the varieties with regard to the quantitative characters of economic importance, it becomes necessary to study them under an array of distinguishable environments. The concept of multiple gene inheritance or of quantitative inheritance is now one of the most important principles of genetics which require refined statistical method. Smith (1944) proposed polygenic concept of quantitative characters and Mather (1949) on the basis of this concept elaborated the statistical method of Fisher *et al*. (1932) to study genetic components of variance as well as environmental variance. As yield is the main object of a breeder, it is important to know the relationship between various characters that have direct and indirect effect on yield. The degree of relationship or association of these characters with yield can be ascertained by correlation studies. Determination of correlation coefficients among the yield and yield components is, therefore, prime importance in selecting suitable plant types and in designing effective breeding programme.

Micro propagation of strawberry has been used in horticultural production since 30 years (Boxus, 1974). Although, some problem stile remaining such as multiple shoots regeneration ability, micropropagation media varied from strawberry cultivars to cultivars. Singh and Pandey (2004) reported that shoot organogenesis varied from strawberry genotypes to genotypes. Some workers reported high concentration of BAP is the best for strawberry micro propagation (Morozova, 2002) while other authors suggested 1.0 mg/l IAA + 1.0 mg/l BAP + 0.05 mg/l GA_3; 0.5 mg/l BA + 0.1 mg/l GA_3 + 0.1 mg/l IBA (Boxus, 1999; Litwińczuk, 2004) and 0.5 mg/l BA + 0.1 mg/l IBA (Bozena, 2001) for strawberry micropropagation. Vegetative and reproductive developments of strawberry are highly sensitive to environmental factors (Durner and Poling, 1988; Le Mie`re *et al.,* 1996; Sønsteby and Nes, 1998; Konsin *et al.,* 2001; Miche`l *et al*., 2007). For better strawberry production photoperiod 10-20 h, day temperature 12-30°C and number of short days 14-28 are essential (Mich e`l *et al.,* 2006). Bangladesh is a subtropical country and here in winter average day-temperature 15-25°C, photoperiod 12-16 h and shorts days about 30-50 days. So strawberry can be growing in Bangladesh during winter. In addition there are no strawberry cultivars that can be commercially cultivated in Bangladesh. In the present investigation

attempts were made to develop more efficient methods of large scale *in vitro* shoot regeneration of three strawberry clones and estimated the field performance in Bangladesh and results will be used subsequent experiment for improvement of strawberry clone through breeding methods.

Materials and Methods

Establishment of aseptic explants

Runner tips were collected from nursery grown stock plants of three strawberry clones (pbgel-01, pbgel-02 and pbgel-03 developed by Plant Breeding and Gene Engineering lab, Department of Botany University of Rajshahi, Bangladesh) and were washed with Tween 80. Afterwards, explants were rinsed several times with sterile distilled water. Surface sterilization was done inside the laminar air flow cabinet by dipping the explants in 0.1% $HgCl_2$ solution (w/v) for five minuets. Sterile runner tips' having terminal buds (3-4 mm) were dissected and cultured in MS medium containing 3% sucrose, 0.8% agar. The pH was adjusted to 5.8 before adding agar and autoclaved. The cultures were incubated in a growth chamber under 16/8 h light/dark cycle at 25±2°C. The aseptic shoots that were obtained after two weeks of cultures were used as a source of explants for subsequent experiments.

Selection of best medium for mass propagation

For mass propagation media selection, aseptic proliferated shoots were cultured on MS media having NAA, BA and Kin used either singly or in combinations. Shoots number, harvestable shoots and the frequency of responded proliferating shoots were recorded. Microshoots were rooted on the half strength of MS medium without growth regulators.

Acclimatization and Field performance of micro propagated plantlets

Three-week-old rooted shoots were removed from the culture tubes, thoroughly washed to remove agar traces and then transferred to plastic pot containing garden soil and cowdang (3:1 v/v). Plants were watered immediately after planting with sprayer and kept under shade covered with transparent plastic sheets made dome to maintain moisture. After one week dome was removed and plants were fully exposed on sunlight.

The transplanted strawberry micro propagated plantlets were evaluated in the experimental field of Plant Breeding and Gene Engineering lab, Department of Botany University of Rajshahi, Bangladesh during 2001-2004. Sixty days old tissue culture derived plantlets and thirty days old runner derived plantlets were sown in field. The experiment was laid out in the randomized complete block design with three replications.

Plantlets were planted at 40 cm × 35 cm distance on 50 cm wide and 350 cm long raised bed. The soil of the experimental plots was specially amended with cowdong and coarse sand (1:1 v/v). Urea-TSP-MP was applied at the rate 75-60-75 kg/ha. The entire dose of TSP, MP and 50% urea was applied as top dressing into two equal installments at 30 and 60 days after planting. Intercultural operations such as earthing up, weeding and mulching were done as required. Field experiments were repeated three times (October-2001 to February-2002; October-2002 to February-2003; October-2003 to February-2004). Nine plants were selected at random from each replication and data were subjected as plant height (PH), no of leaf (NL), canopy size (CZ), no of runner (NR), no of crown (NC), no of fruit per plant (NF) and average fruit weight per fruit (AFW). Most of the traits were measured at three months after plantation where as the NF and AFW were measured at after four months after plantation.

Data analysis

Data were analyzed by some biometrical techniques developed by Mather (1949), Hayman (1958), De-wey and Lu (1959) and Allard (1960). Means were separated with Duncan's multiple range test ($P<0.05$) where appropriate. Correlation between seven agronomic traits and some genetic parameters were also estimated. All the statistical analysis was performed by using MS excel and MSTAT-C and SAS software.

Results

Sterilized runner tips cultured in MS medium without any growth regulators. After two weeks aseptic runner tips were transferred to multiple shoot induction media. To evaluate the best media formulations for mass propagation of three strawberry clones were tested on 18 different treatments (Table 1). The result revealed that tested 18 different micropropagated media were statistically different from each other for number of shoots and harvestable shoots production.

Among the various plant growth regulators supplements used, the best response towards multiple shoot regeneration was observed from runner tip explants on MS medium supplemented with 0.5 mg/l BA. This combination showed the best performance of shoot proliferation. The highest number of shoots and the highest number of harvestable shoot per culture were also recorded from this medium. % of culture response was best in low BA concentration containing media. Analysis of variance (Table 2) revealed that treatments source was highly significant for both number of shoots and harvestable shoot per culture. Replication and clones were non significant for number of shoots and number of harvestable shoots production where as clones were significant at 1% significant level. These results indicated that considerable variation was present among the mass propagation media used in this study and clones were not more diverse for shoot and harvestable shoot production.

The runner tip cultured in MS medium supplemented with NAA (3-3.5 mg/l) produce callus without proliferating roots and shoots (Fig.1B). Callus also formed when low concentration of 2,4-D present in culture media (Fig. 1C).

Proliferation of shoots from the runner tip explants was remarkably influenced by the types and concentration of the cytokine used. The BA at most of the cases was comparatively more effective than that of Kin in proliferation of shoots. With the decreases in BA concentrations from 4.0 – 0.1 mg/l the percentage of explants showing proliferation and number of shoot per culture increased gradually.

Mean performance, analysis of variance, some genetic parameters, correlation and path coefficient were estimated for seven agronomic important traits of three strawberry clones and results are presented in Table 3-6 nificantly differences were found among the studied colons in regards to yield contributing traits such as NL, NC, NF and AFW. Mean was not significantly difference among the studied years. On the other hand mean was significantly different in the traits NL, NC, NF and ATW among the tissue culture derived plantlets and runner derived plantlets. This result is in agreement with Damiano (1980), Lis *et al.* (1995), Lopez-Aranda *et al*. (1997) and Litwińczuk (2004).

Analysis of variance revealed that the strawberry clones (C) showed highly significant difference in most of the traits except PH and CZ (Table 3). The year (Y) showed significant difference for the traits NR, NC and NF. On the other hand type of plantlets (T) shows highly significant difference for all the studied traits except NR. C×Y show significance difference only the trait NL. Interaction C×T of explants and C×Y×T of explants show no significance difference all the studied traits. On the other hand Y×T of explants show significance difference only the traits NL and NR.

Table 2. Analysis of variance for number of shoots and harvestable shoots per culture of three strawberry clones.

Source of variance	df	No of shoots per culture	No of harvestable shoots per culture
Replications	2	ns	ns
Treatments	17	***	***
Clones	2	ns	ns
Treatments× Clones	34	**	**

Significance of replication, treatments, clones and treatments ×clones is given: ns: non significant,
**P<0.01 and ***P<0.001

Table 3. Field performance of tissue culture derived plantlets on field condition in Bangladesh.

	PH	NL	CZ	NR	NC	NF	AFW
Clone (C)†							
pbgel-01	10.33[a]	51.54[a]	22.45[a]	5.07[a]	4.39[a]	11.61[a]	8.35[c]
pbgel-02	11.19[a]	38.67[b]	23.34[a]	5.19[a]	2.92[b]	9.96[a]	11.90[b]
pbgel-03	11.37[a]	42.00[b]	25.87[a]	6.20[a]	2.61[b]	7.24[b]	17.22[a]
Year (Y) †							
2001-2002	10.72[a]	42.45[a]	24.80[a]	4.88[b]	3.17[a]	9.73[a]	11.96[a]
2002-2003	11.50[a]	45.17[a]	23.36[a]	4.82[b]	2.99[a]	8.96[a]	12.57[a]
2003-2004	10.68[a]	44.58[a]	23.50[a]	6.76[a]	3.76[a]	10.12[a]	12.93[a]
Type of plantlets (T) †							
RDP	11.91[a]	48.90[a]	26.85[a]	5.26[a]	4.22[a]	11.00[a]	13.23[a]
TDP	10.02[a]	39.24[b]	20.93[a]	5.72[a]	2.40[b]	8.21[b]	11.75[b]
LSD	2.173	5.834	9.941	1.851	1.238	2.057	2.518
CV%	11.94	7.98	25.27	20.32	22.69	12.91	12.15
Analysis of variance							
C	ns	***	ns	**	***	***	***
Y	ns	ns	ns	***	**	*	ns
T	***	***	**	ns	***	***	**
C ×Y	ns	**	ns	ns	ns	ns	ns
C × T	ns	ns	ns	ns	ns	ns	ns
Y × T	ns	***	ns	ns	*	ns	ns
C× Y ×T	ns	ns	ns	ns	ns	ns	ns

PH= Plant height, NL= Number of leaf, CZ= Canopy size, NR=Number of runner, NC= Number of crown, NF= Number of fruit, AFW= Average fruit weight, NS= non significant, *= Significant at 5% significant level. † = Value followed by different letter are significantly different according to DMRT-test at P<0.05 level.

Table 4. Estimation of genetic parameters of seven agronomic traits in three strawberry clones.

Genetic parameters	PH	NL	CZ	NR	NC	NF	AFW
σ^2g	1.283	263.960	0.922	1.885	5.395	28.817	118.558
σ^2p	2.998	276.323	36.817	3.130	5.952	30.354	120.860
σ^2e	1.715	12.363	35.895	1.245	0.557	1.537	2.302
GCV	10.329	36.866	4.020	25.008	70.193	55.896	87.184
PCV	15.789	37.719	25.399	32.225	73.727	57.368	88.027
ECV	11.942	7.978	25.079	20.324	22.553	12.909	12.149
H^2b	0.428	0.955	0.025	0.602	0.906	0.949	0.981
GA	1.927	41.286	0.395	2.770	5.750	13.599	28.039
GA%	17.568	93.682	1.654	50.458	173.753	141.603	224.510

PH= Plant height, NL= Number of leaf, CZ= Canopy size, NR=Number of runner, NC= Number of crown, NF= Number of fruit, AFW= Average fruit weight, σ^2g =Genotypic variability, σ^2p = Phenotypic variability, σ^2e = Environmental variability, GCV = Genotypic coefficient of variability, PCV = Phenotypic coefficient of variability, ECV = Environmental coefficient of variability, H^2b = Heritability, GA = Genetic advanced of mean, GA% = Genetic advanced %of mean,

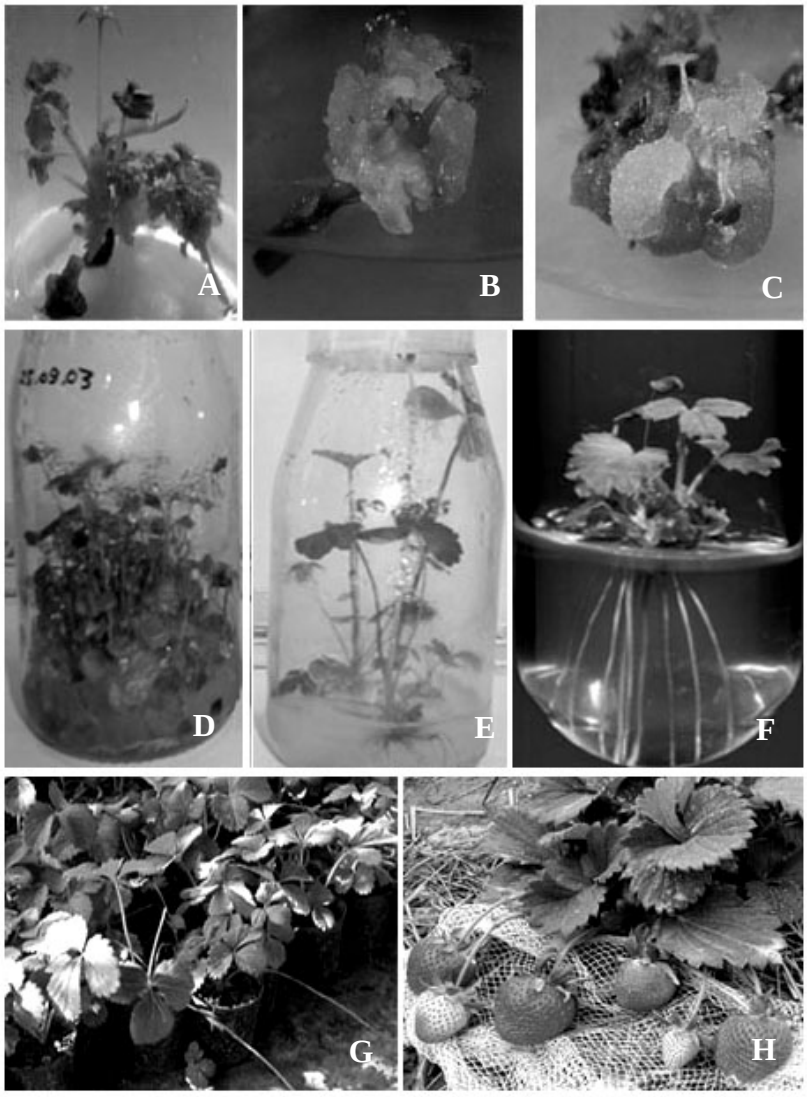

Fig. 1. A. shoots multiplication on 0.5 BA+0.15 kin after 4 weeks of culture.
B. Callus formation on 2, 4-D containing medium after 4 weeks of culture.
C. Callus formation on NAA containing medium after 4 weeks of culture.
D. Micro shoots formation on 0.5 BA after 4 weeks of sub culture.
E. Shoot elongation on GA_3 containing medium after three weeks of culture.
F. Rooting on 1/2 MS medium after three weeks of culture.
G. Well established plants on pots after acclimatization.
H. Micro propagated plantlets produce fruits in field.

The result revealed that estimated phenotypic variance (Table 4) was higher than genotypic and environmental variance. High values of phenotypic coefficient of variation (PCV) than genotypic coefficient of

variation (GCV) indicated considerable environmental influence upon the phenotypic expression all the studied traits. High value of genetic coefficient of variation was to be found for AFW, NC and NF suggesting higher degree of genetic variation for these traits.

High heritability estimates in broad sense was the higher for AFW followed by NL, NF and NC. High value of heritability together with high genetic advanced % of mean was recorded for AFW, NF, NC and NL. Genotypic coefficient of variation, heritability and genetic advanced % of mean were found to be higher for AFW and NC.

Genotypic, phenotypic and environmental correlation co efficient is presented in Table 5. Phenotypic correlations among the different pairs of character exhibit non significant correlation. AFW was positively correlated with PH, CZ, NR and NF. Among the different pairs of characters CZ exhibit positive significant genotypic correlation with CZ, NR and AFW, where as CZ also exhibit negative significant correlation with NC and NF. Environmental correlation coefficient also exhibit non significant correlation among the studied all characters pairs.

The results from Table 6 reveals NF had the highest direct positive effect on yield for all the phenotypic, genotypic and environmental level. On the other hand NC exhibit lowest direct negative effects on yield for phenotypic and genotypic level where as low negative direct effect for environmental level was recorded in NL.

Discussion

The method of strawberry micropropagation was described about thirty years ago (Boxus, 1999). Adventitious shoots occur spontaneously in *in vitro* culture is common in strawberry (Jemmali *et al*., 1992; Rugini and Orlando, 1992; Jemmali *et al*., 1994a; Jemmali *et al*., 1994b; Damiano *et al*., 1997; Boxus, 1999). The adverse method of adventitious shoots suppression without retardation of axillary shoots' growth is still not elaborated. On the other hand the micropropagation of strawberry by adventitious shoots should be more effective, easier and cheaper than by auxiliary ones as the rate of multiplication of such shoots is often much higher (Jemmali *et al*., 1994a; Jemmali *et al*., 1994b).

Table 5. Correlation co-efficient of Phenotypic genotypic and environmental.

Traits		PH	NL	CZ	NR	NC	NF	AFW
PH	*p*		-0.129	0.118	0.076	-0.124	-0.108	0.123
	g		-0.176	0.525*	0.159	-0.202	-0.181	0.172
	e		-0.106	0.085	-0.011	0.007	0.044	0.111
NL	*p*			-0.049	-0.050	0.137	0.092	-0.107
	g			-0.411	-0.067	0.158	0.106	-0.104
	e			0.068	0.010	-0.150	-0.191	-0.226
CZ	*p*				0.094	-0.090	-0.142	0.102
	g				0.852***	-0.619**	-0.673**	0.765***
	e				-0.017	0.010	-0.170	-0.165
NR	*p*					-0.098	-0.134	0.136
	g					-0.130	-0.179	0.175
	e					-0.011	0.009	0.016
NC	*p*						0.139	-0.142
	g						0.147	-0.149
	e						0.030	-0.031
NF	*p*							0.061
	g							0.391
	e							0.251

PH= Plant height, NL= Number of leaf, CZ= Canopy size, NR=Number of runner, NC= Number of crown, NF= Number of fruit, AFW= Average fruit weight,
* , ** and *** significant at 5%, 1% and 0.1% significant level, respectively
p= Phenotypic correlation co-efficient, *g*= Genotypic correlation co-efficient and *e*= Environmental correlation co-efficient

Indra and Uppeandra (2000) reported multiple shoot regeneration from Indian wild strawberry using MS supplemented with 4.0 mg/l BA and 0.1mg/l NAA. Some workers also reported shoot regeneration in strawberry using MS medium containing BA also of in combination with Kin (Lee and de Fossard, 1977; James and Newton, 1977; Sobczykiewicz, 1980; Lis, 1990; Boxus, 1999; Neeru *et al*., 2000; Mereti *et al*., 2003). However, the results of the present investigation slightly differed with that of the previous works. Our results indicated that, low concentrations of BA alone or with kin were found suitable for shoot initiation and further multiplication. This difference may be attributed by the difference of genotype and physiological condition of the explants.

A crown production phenomenon is statistically difference between the runner derived and tissue culture derived plantlets. Runner derived plantlets produced more crown than tissue culture derived plantlets. Similar

result was also obtained by Litwińczuk (2004). Runner derived plants produce big size berry than tissue culture derive plants (Litwińczuk, 2004). In our experiment we found average fruit weight is more in runner derived plantlets than tissue culture derived plantlets.

Studies on variability, heritability, phenotypic and genotypic co-efficient of variation helps to identify yield relating characters for improving crops. Identification of genotypes with high variability and heritability for describe characters are per-requisite in the development of new varieties with increased yield potential. Information on the nature and magnitude of variation in the populations, the extent of environmental influence on the expression of characters is necessary for fruitful gain in breeding program. The genetic parameters also help in the prediction of possible genetic advance through selection based on phenotypic value.

In the present study the estimation of genetic parameter revealed that the genotypic variance followed the same trend of phenotypic variance for all the characters, indicating that phenotypic variability may be considered a reliable measure of genetic variability. The low value of environmental variance then genotypic and phenotypic variance for all the traits except PH and CZ indicated that the environmental influences were negligible for the expression of these traits. Genotypic coefficient of variation for majority of the traits was quit close to the estimates of phenotypic coefficient of variation indicating negligible environmental role and the genotypic performance appeared to the environment for the fullest phenotypic expression of the traits. High genotypic co efficient of variability as well as phenotypic co efficient of variability percentage was observed for AFW, NC and NF in all the analysis. These results suggest that the greater variability for these traits among the variance was due to genetic causes which are less affected by environment and hence could be improved through selection.

Heritability estimates in broad sense were relatively high for almost all the traits studied. Although high heritability estimates have found to be helpful in making selection of superior genotypes on the basis of phenotypic performance. Johnson *et al*. (1955) suggested that heritability estimates along with genetic gain were more useful in predicting the effect for selecting the best individual.

Table 6. Phenotypic, genotypic and environmental path co-efficient analysis showing direct (bold) and indirect effect of various characters on yield in strawberry.

		PH	NL	CZ	NR	NC	NF	Yield
PH	*p*	**0.0772**	0.0086	0.0067	0.0074	0.0116	0.0117	0.1232
	g	**0.4510**	0.0689	-0.8146	0.1859	0.1391	0.1412	0.1715
	e	**0.0945**	0.0191	-0.0108	-0.0002	-0.0004	0.0085	0.1107
NL	*p*	-0.0100	**-0.0665**	-0.0028	-0.0049	-0.0128	-0.0100	-0.1070
	g	-0.0794	**-0.3916**	0.6378	-0.0789	-0.1087	-0.0830	-0.1038
	e	-0.0100	**-0.1804**	-0.0086	0.0001	0.0094	-0.0367	-0.2262
CZ	*p*	0.0091	0.0033	**0.0568**	0.0092	0.0084	0.0154	0.1022
	g	0.0237	0.0161	**0.5505**	0.0957	0.0263	0.0526	0.7649
	e	0.0080	-0.0122	**-0.1269**	-0.0002	-0.0006	-0.0328	-0.1647
NR	*p*	0.0058	0.0033	0.0053	**0.0978**	0.0091	0.0146	0.1359
	g	0.0717	0.0264	-0.3207	**0.1689**	0.0894	0.1397	0.1754
	e	-0.0010	-0.0017	0.0022	**0.0141**	0.0007	0.0018	0.0161
NC	*p*	-0.0096	-0.0091	-0.0051	-0.0096	**-0.0932**	-0.0151	-0.1417
	g	-0.0911	-0.0618	0.9595	-0.1517	**-0.6889**	-0.1152	-0.1492
	e	0.0006	0.0270	-0.0013	-0.0002	**-0.0631**	0.0058	-0.0312
NF	*p*	-0.0083	-0.0061	-0.0080	-0.0131	-0.0129	**0.1090**	0.0606
	g	-0.0815	-0.0416	0.0435	-0.2091	-0.1016	**0.7812**	0.3909
	e	0.0042	0.0344	0.0216	0.0001	-0.0019	**0.1928**	0.2512

PH= Plant height, NL= Number of leaf, CZ= Canopy size, NR=Number of runner, NC= Number of crown, NF= Number of fruit, AFW= Average fruit weight
Bold value indicate direct effect and non bold value indicate indirect effect
p= Phenotypic path co-efficient, *g*= Genotypic path co-efficient and *e*= Environmental path co-efficient

The correlation co-efficient between AFW and its component traits and among various components themselves were estimated at genotypic, phenotypic and environmental levels. It was revealed that in most of the cases, the values of genotypic correlation co-efficient were higher than the corresponding phenotypic and environmental correlation co-efficients indicating less pronounced environmental effect. Lower phenotypic correlation coefficients than genotypic correlation coefficients indicate that both environmental and genotypic correlations in those cases act in same direction and finally maximize their expression at phenotypic level. Among the studied different traits AFW was found to be positively and significantly associated at genotypic levels with CZ and AFW was also found positive association at phenotypic, genotypic and environmental level with PH and NR. High negative significant association of CZ with NC and NF were found only in genotypic level.

Path coefficient analysis at genotypic, phenotypic and environmental level was estimated. Path coefficient values based on phenotypic correlation revealed that PH, CZ, NR and NF had direct positive effect towards AFW also having positive correlation with AFW. Therefore, proper attention should be taken on above characters for the improvement of AFW. The present investigation on correlation and path analysis suggests that during selection more emphasis should be given CZ and NF, since these characters, have high correlation and high direct effect on AFW.

Conclusion

In conclusion it can be standard that low concentration of BA is more effective for mass propagation of the studied three strawberry clones. Tissue culture derived plantlets are more suitable for propagation because they produced more runners in field condition than plants obtained by standard propagation and runner derived plants is suitable for fruit production.

References

Allard, R.W. (1960). Principles of Plant Breeding. Jhon wiley and Sons, Inc, New York

Boxus, P. (1999). Micropropagation of strawberry via axillary shoot proliferation. In: Plant Cell Culture Protocols. Methods in Molecular Biology. Part III. Plant Propagation In Vitro. Hall R. D. (ed.) Humana Press Inc., Totowa NJ, 111: 103-114

Boxus, P. (1974). The production of strawberry plants by in vitro micropropagation. J. Hortic. Sci. 49: 209-210

Bozena Borkowska. (2001). Morphological and phylosiological characteristics of micropropagationed strawberry plants rooted in vitro or ex vitro. Scienta Horticulture 89: 195-206.

Damiano, C. (1980). Strawberry micropropagation. In: Proceedings of the conference on nursery production of fruit plants through tissue culture: Application and feasibility. Beltsville, Maryland, USA, 93-101

Damiano, C., Monticelli, S. and Corazza, L. (1997). Somaclonal varaibility and in vitro regeneration of strawberry. Acta Hort. 447: 87-93

Damiano, C. (1980). Strawberry micropropagation . W: Proc. Conf Nursery production of fruit plants through tissue culture. Applications and feasibility. April 21-23, Beltsville MD, p. 11-22

De-Wey, D.R. and Lu, K.H. (1959). A correlation and path-coefficient analysis of components of orested wheat grass and production. Agron J, 51: 511-518

Drew, R.A., Herrington, M.E., Greber, R.S. and Duncalfe F. (1986). Tissue culture and subsequent field evaluation of strawberry. Queen.J. Agric. Anim. Sci. 43: 91-96

Durner, E.F. and Poling, E.B. (1988). Strawberry developmental responses to photoperiod and temperature: a review. Adv. Strawberry Prod. 7: 6–15

Fisher, R.A., Lmmer, F.R. and Ledin, O. (1932). The genetically interpretation of statistics of the third degree of study of quantitative inheritance. Genetics, 17: 107-128

Hayman, B.I. (1958). The separation of epistatic from additive and dominance variation in generation means. Heredity, 12: 370-390.

Indra, D., Bhatt and Uppeandra Dhar. (2000). Micropropagation of Indian wild strawberry. Plant cell and Organ Culture 60(2): 83-88

James, D.J. and Newton, B. (1977). Auxin: Cytokinin Interactions In The In Vitro Micro-Propagation Of Strawberry Plants. Acta Hort. (ISHS) 78: 321-332

Jemmali, A., Boxus, P., Dekegel, D. and Van Heule, G. (1994a). Occurrence of spontaneous shoot regeneration on leaf stipules in relation to hyper flowering response in micropropagated strawberry plantlets. In Vitro Plant Cellular and Developmental Biology. 309: 192-195

Jemmali, A., Boxus, P., Kevers, C. and Gaspar, T. (1994b). Flowering abundance of strawberry on dependence of the number of subcultures in vitro. Relationship with growth, rooting and peroxidase activity. Physiology, Growth and Development of Plants in Culture. P.J. Lumsden, J.R. Nicholas, W.J. Davies eds., Kluwer Acad. Publ. Dordrecht: 356-362

Jemmali, A., Boxus, P., Kinet, J.M., Hayashi, M. (ed.)., Kano, A. (ed.), Goto, E. (1992) Are strawberry plantlets arising from adventitious stipule buds also true to type? Acta Hort. 319: 171-176

Jemmali, A., Boxus, P., Kevers, C. and Gaspar, Th. (1995). Carry-over of morphological and biochemical characteristics associated with hyperflowering of micropropagated strawberries. J. Plant Physiol. 147: 435-440

Johnson, H.W., Robinson, H.F. and Comstock, R.E. (1955). Estimates at genetic and environmental variability in Soybean. Agron J 47(7): 314-318

Konsin, M., Voipio, I. and Palonen, P. (2001). Influence of photoperiod and duration of short-day treatment on vegetative growth and flowering of strawberry (Fragaria x ananassa Duch.). J. Hort. Sci. Biot. 76: 77–82

Lee, E.C.M. and de Fossard, R.A. (1977). Some Factors Affecting Multiple Bud Formation of Strawberry (X Fragaria Ananassa Duchesne) In Vitro. Acta Hort. (ISHS) 78: 187-196

Le Mie`re, P., Hadley, P., Darby, J. and Battey, N.H. (1996). The effect of temperature and photoperiod on the rate of flower initiation and the onset of dormancy in the strawberry (Fragaria x ananassa Duch.). J. Hort. Sci. Biot. 71: 361–371

Lis, E., Sowik, I. and Chlebowska, D. (1995). Mikrorozmnażanie truskawki – wpływ warunków *in vitro* na wzrost roślin w glebie. Materiały Ogólnopolskiej Konferencji Naukowej 'Nauka Praktyce Ogrodniczej' z okazji XXV-lecia Wydzialu Ogrodniczego Akademii Rolniczej w Lublinie [Micropropagation of strawberries – effect of *in vitro* conditions on their field performance], 207-210. [in Polish]

Lis, E.K. (1990). In Vitro Clonal Propagation of Strawberry From Immature Achenes. Acta Hort. (ISHS) 280: 147-150

Litwińczuk W. (2004). Field performance of 'senga sengana' strawberry plants (*Fragaria* × *ananassa* duch.) Obtained by runners and *in vitro* through auxiliary and adventitious shoots Electronic Journal of Polish Agricultural Universities, Horticulture 7: (1)

Lopez-Aranda, J.M., Medina, J.J., Barcelo, M., Lopez-Medina, J., Pliego-Alfaro, F. (1997). Field behavior of micropropagated strawberry plants. Proc. Third Int. Strawberry Symp. van der Scheer H. A. T., Lieten F., Dijkstra J. (eds) Acta Hort. 439: 353-357

Mather, K. (1949). Biometrical genetics. Dover Pub. INC. N.Y.

Mereti, M., Grigoriadou, K., Levantakis, N. and Nanos, G.D. (2003). In vitro rooting of strawberry tree (*Arbutus unedo* l.) In medium solidified by peat - perlite mixture in combination with agar. Acta Hort. (ISHS) 616: 207-210

Miche`l J. Verheul, Anita Sønsteby. and Svein O. Grimstad. (2007). Influences of day and night temperatures on flowering of Fragaria x ananassa Duch., cvs. Korona and Elsanta, at different photoperiods Scientia Horticulturae 112: 200-2006

Miche`l J. Verheul, Anita Sønsteby. and Svein O. Grimstad. (2006) Interactions of photoperiod, temperature, duration of short-day treatment and plant age on flowering of *Fragaria*× *ananassa* Duch. Cv. Korona. Scientia Horticulturae, 107: 164-170.

Morozova, T. (2002). Genetic stability of Pure lines of *Fragaria vesca* L in micropropagation and long-term storage in vitro. Acta Hort, 567: 85-87

Neeru Sood, Ranjan Srivastava, Singh O.S. and Gosal S.S. (2000). Enhancing micropropagation efficiency of strawberry using bandage in liquide media. Jour. Appl. Hort. 2(2): 92-93

Rugini, E. and Orlando, R. (1992). High efficiency shoot regeneration from calluses of strawberry (*Fragaria* × *ananassa* Duch.) stipules of in vitro cultures. J. Hort. Sci. 67 (4): 577-582

Singh, A.K. and Pandey, S.N. (2004). Genotypic variation among strawberry cultivars for shoot organogenesis. Acta Hort. (ISHS) 662: 277-280

Smith, H.H. (1944). Recent studies on the inheritance of quantitative characters in plants. Bot Rev, 10: 349-382

Sobczykiewicz, D. (1980). Heat treatment and meristem culture for the production of virus-free strawberry plants. Acta Hort. (ISHS) 95: 79-82

Sønsteby, A. and Nes, A. (1998). Short day and temperature effects on growth and flowering in strawberry (Fragaria x ananassa Duch.). J. Hort. Sci. Biot. 73: 730–736

Swartz, H.J., Lindstrom, J.Y., Fiola, J.A. and Zimmerman, R.H. (1987). The use of tissue culture propagation of strawberry in the United States. In: *In vitro* culture of Strawberry Plants (Eds.Boxus P. and Larvor M.P.) C.E.C.Luxemburg, p. 17-20

(Received 03 April 2008; accepted 20 May 2008)

Table 1. Effects of BA, Kin and NAA on shoot multiplication of three strawberry clones.

MS+Plant growth regulators (mg/l)			No. of shoot per culture			No. of harvestable shoot per culture			% of culture response		
BA	**NAA**	**Kin**	**pbgel-01**	**pbgel-02**	**pbgel-03**	**pbgel-01**	**pbgel-02**	**pbgel-03**	**pbgel-01**	**pbgel-02**	**pbgel-03**
0.1			20.4 ±1.3^{f}	20.5±0.5^{d}	21.2±0.4^{d}	13.4±1.3^{d}	14.1 ±2.1^{c}	12.4±1.5^{ef}	92	91	93
0.5			50.1±1.0^{a}	49.7±0.6^{a}	50.4±1.2^{a}	34.2±2. 1^{a}	33.5±1.1^{a}	33.8±1.4^{a}	97	96	95
1.0			24.3 ±0.9^{de}	23.9±2.4^{d}	24.1±2.2^{d}	16.6±0.5^{c}	15.9±1.7^{c}	16.5±0.7^{cd}	96	98	96
1.5			13.6±0.5^{g}	14.2±1.1^{e}	12.5±1.1^{e}	7.5±0.3^{ef}	6.7±1.1^{efg}	7.3 ±1.2^{gh}	95	94	92
3.0			12.8±0.8^{gh}	13.8±0.9^{ef}	13.8±1.7^{e}	7.4±0.6^{ef}	6.9±0.8^{def}	8.0±0.9^{gh}	95	94	92
4.0			29.9±1.3^{c}	30.5±1.0^{c}	29.5±2.1^{c}	7.6±0.7^{ef}	7.8±1.1^{def}	7.9±1.4^{gh}	93	93	90
0.1		0.15	32.8±1.4^{b}	34.8±0.8^{b}	34.1±1.2^{b}	18.2±1.3^{c}	19.8±0.9^{b}	18.9±1.3^{c}	92	90	90
0.5		0.15	22.6±1.0^{def}	23.1±0.8^{d}	22.3±1.2^{d}	24.7±2.4^{b}	23.2±2.2^{b}	25.9±1.9^{b}	92	90	90
1.0		0.15	19.6±0.5^{f}	20.5±1.1^{d}	21.8±0.3^{d}	15.9±1.5^{cd}	14.7±2.3^{c}	13.0±1.4^{de}	89	85	86
1.5		0.15	25.6±1.1^{d}	27.9±0.6^{c}	24.8±1.2^{d}	12.1±0.5^{d}	11.5±1.5^{cd}	12.3±1.7^{ef}	89	85	86
0.1		0.5	22.1±0.8^{ef}	21.5±0.8^{d}	20.8±0.6^{d}	8.2±1.6^{e}	8.8±0.8^{def}	9.7±1.4^{efg}	81	80	80
0.5		0.5	10.9±1.2^{ghi}	10.7±0.9^{efg}	10.5±0.5^{ef}	12.7±1.1^{d}	11.5±2.1^{cd}	12.2±2.8^{ef}	87	86	85
1.0		0.5	7.4±0.6^{jk}	7.6±0.9^{ch}	8.2±0.8^{f}	5.5±1.7^{efg}	5.5±1.5^{def}	6. 4±0.7^{ghi}	81	80	80
1.5		0.5	9.2±0.8^{ij}	9.5±1.5^{ch}	9.6±1.9^{ef}	8.2±0.3^{e}	9.7±1.4^{de}	8.5±1.5^{fgh}	78	75	75
0.1	0.5		10.2±0.8^{hij}	10.5±0.5^{fg}	10.9±0.8^{ef}	5.2±0.5^{efg}	4.3±0.4^{fg}	5.5±0.5^{hi}	42	40	45
0.1	1.0		9.4±0.7^{ij}	9.2±1.1^{gh}	9.9±0.8^{ef}	4.8±0.8^{fg}	4.9±0.5^{efg}	4.8±0.6^{hi}	53	55	50
0.5	0.5		10.1±0.7^{hij}	10.2±0.8^{gh}	10.2±1.3^{ef}	4.6±0.3^{fg}	4.6±0.3^{efg}	4.4±0.4^{hi}	60	62	60
0.5	1.0		6.2±1.2^{k}	6.6±1.5^{h}	7.3 ±1.4^{f}	2.1±0.5^{g}	2.1±0.5^{g}	2.1±0.5^{i}	40	45	45

Values followed by different letters are significantly different according to DMRT-test at $P<0.05$ level