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Somaclonal Variation in Potato (*Solanum tuberosum* L.) Using Chemical Mutagens

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Abstract

An experiment was conducted with three popular potato varieties *viz*. Cardinal, Diamant and Asterix to create somaclonal variation in potato. The chemical mutagens *viz*. Ethyl methane sulphonate (EMS), Methyl methane sulphonate (MMS), 5-Bromo Uracil (BU) and 2,4-D were used in three different concentration (1.0, 2.0 and 4.0 mg/L). Among them only 2,4-D regenerated callus in potato. Higher concentration (4.0 mg/L) of 2,4-D showed variant type of callus, which regenerated abnormal plantlet and some of the plantlets died within 45 days after inoculation. The higher concentration (4.0 mg/L) of EMS, MMS and BU showed huge abnormality on *in vitro* regeneration in all three varieties of potato. Thin stem, deformed shoot development and very less leaf formation were observed in 2.0 mg/L and 4.0 mg/L of EMS, MMS and BU. Due to toxic effect some of the plantlets died. The mutagen treated variants were acclimatized in plastic tray and subsequently in the field condition. It was noticed that, only 37.16% plants survived in natural field condition. Morphological characterization and yield potentiality of all somaclones were studied. It revealed that only one variants *viz*. SVP-53 showed higher yield as compared with two check varieties. The first generation mini tubers were kept for further research.

Keywords: Somaclonal variation, chemical mutagen, Potato

1. Introduction

Potato (*Solanum tuberosum* L.) is an important vegetable crop in Bangladesh which has versatile use in our daily consumption as well as industrial purpose. The total area under potato cultivation is near about 0.6 million hectares and the average yield was 19.69 t/ha, (BBS, 2012). The development of high yielding, more starch enriched, disease resistant varieties is needed for sustainable potato production. In any crop improvement program, genetic diversity has been considered as an important factor for developing cultivar with increased yield and wider adaptability in adverse environmental condition. Till now, only few potato varieties have been cultivated throughout the country which has less genetic variability.

Genetic recombination and variability creation by conventional hybridization method is very difficult in potato due to its shy flowering habit and vegetative mode of propagation. Hence, advanced tissue culture technology can be applied in potato for creation of novel variation. Larkin and Scowcroft (1981) adopted the term

"somaclonal variation" to describe the genetic variations occurring *in vitro* cultured cells, tissues and plants. It has the potentiality to assist plant breeders to create new variation for crop improvement. Many varieties have been developed in sugarcane, mustard, rice, *Apium* etc. (Karp, 1995) by using this technique.

Different factors are identified which influence whether or not variation will be produced and how much variation will be created. It depends on the genetic constitution of source materials, the nature of mutagenic agents, type of explants, etc. Potato being a vegetatively propagated crop, its traditional sexual true breeding approaches are limited. On the other hand, its polyploid genomic structure gives an opportunity to work on somaclonal variation methods for its improvement. Hence, the experiment was designed to create somaclone using different chemical mutagens to create economically important traits in potato.

2. Materials and Methods

2.1. Callus induction and plantlet regeneration and plantlet acclimatization

The experimental materials were three popular varieties *viz.*, Cardinal, Diamant and Asterix which were collected from the Tuber Crops Research Centre (TCRC), Bangladesh Agricultural Research Institute (BARI), Gazipur. Fresh sprouts were used as explants. Those explants were washed with running tap water followed by dipping in 70% ethanol for 1 minute, then rinsed with double distilled water and dipped in 0.1% $HgCl_2$ for 2-3 minutes for surface sterilization. The explants were cultured in Murashige and Skoog (MS) medium (1962) supplemented with three concentrations *viz.*, 1.0, 2.0 and 4.0 mg/L of each chemical mutagen, EMS (Ethyl Methane Sulphonate), MMS (Mthyl Methane Sulphonate), BU (5-Bromo-Uracil) and 2, 4-D. All these works were done under aseptic condition under Laminar Airflow Hood to avoid contamination. The culture vials were incubated on 3000 lux for 16:8 hour photoperiod at a temperature of 23±1 ^{o}C.

The experiment was laid out in a completely Randomized Design (CRD) having two factors (variety and treatment) with three replications. Data were analyzed using MSTAT-C statistical program. The differences among the means were compared by the least significant different test at 5% level. Data were recorded on days to callus induction, callus size, callus weight, days to shoot initiation, days to root induction, regeneration of normal and abnormal plantlets. Autocleaved garden soil, sand and cowdung were mixed in the ratio of 1:2:1 and were placed in a plastic tray having 4×4 cm small chamber. The plantlets of 40-50 days having well developed roots were removed from culture vial and the roots were washed gently on running tap water. It was immediately transplanted into plastic tray and irrigated with fine spray of water and were kept in a shaded place. After 10-15 days the plantlets were transferred to the main field. Chemical mutagenic treated plantlets were named as SVP (somaclonal variant of potato) and numbering was done chronologically like SVP-01, SVP-02 etc.

2.2. Morphological characterization of somaclonal variants

The mutagen-treated somaclonal variants were nourished under field condition. The whole experimental materials were covered by mosquito net to protect from viral infection through insect. All inter cultural operations were done whenever needed. Good crop management practices were followed to produce minitubers (1st generation tuber) from the different SVP genotypes. Data were recorded on plant height, stem colour, leaf colour, leaf shape, number of tubers per plant, tuber size, tuber weight and yield per plant.

3. Results and Discussion

The chemical mutagens *viz*. EMS, MMS, 5-BU and 2, 4-D were used to create somaclone in three potato varieties - Cardinal, Diamant and Asterix. It was observed that, out of them only 2, 4-D had the ability to induce callus in different potato cultivars.

3.1. Callus induction and somaclonal variant creation in potato

Callus induction and its morphological parameters in different the varieties are presented in Tables 1 and 2. It was observed that simple MS medium had no ability to induce callus in all the three varieties. MS media supplemented with 4.0 mg/L of 2, 4-D required less time to induce calli in all the genotypes. The maximum time (10.0 days) was noticed in Asterix with the treatment MS +1.0 mg/L of 2, 4-D. Vigorous and robust shoot initiation was noticed in all three varieties in MS+2.0 mg/L of 2, 4-D. (Fig. 1).

The biggest size of callus (2.2cm) was observed in the varieties Cardinal and Asterx at 45 days after initiation. Although, the size of calli was the same in both the genotypes but the final weight of calli at 45 days showed little difference between Cardinal and Asterix. Shoot iniciation, root iniciation, normal and abnormal variant regeneration data are presented in Table 2. Shoot per plantlet increased gradually in 30 days and 45 days but healthy, abnormal and deformed shoot formation were observed (Fig. 2) in the higher concentration (4.0 mg/L) of 2, 4-D. The maximum no. of 13.2 shoots were found in the treatment MS+2.0 mg/L 2, 4-D in the variety Asterix at 45 days. Roots per variant were the highest (17.9) in Cardinal with the same treatments. Root per plantlet showed positive correlation with shoot per plantlet. Ehsanpour *et al*. (2007) obtained calli from *in vitro* grown potato leaf segment on MS medium containing 2, 4-D, NAA, Kinetin and yeast extract. They reported the changed DNA pattern as the source of genetic variation.

However, they mentioned that, somaclonal variation could be used for selection of potato calli toward desirable traits, such as salt or drought stress tolerance. Somaclonal variation in potato meristem culture was also reported by Rosenberg *et al*. (2010). They showed that meristem clone differed in yield, number and weight of tubers and late blight resistance. In addition, they reported deviation from true to type in morphological characteristics of meristem clones. This finding was in conformity with our present observations. Patricia *et al*. (2004) reported that 1.65 mM of piclorm and 11.5 mM of 2, 4-D created somaclonal variation in potato.

3.2. Effect of mutagen on in vitro regeneration

The effects of chemical mutagen on *in vitro* regeneration in potato are presented in Table 3. Direct shoots were developed from the explants treated with the mutagens. Toxic effect was observed in higher concentration of mutagens and took more time to shoot initiation in all three varieties. In some cases, regenerated variants died within 15 days of culture. Among the mutagens, BU had more corrosive effect on the plantlets. However, the individual effect of each treatment is given below. Days to shoot initiation were the maximum (13.0) in the treatment MS+ 4.0 mg/L of BU in Cardinal. It was the minimum (4.5) in the treatment MS+1.0 mg/L of EMS in the variety Diamant. Number of shoot per plantlet was the highest (19.6) at 45 days in treatment MS+ 4.0mg/L of MMS in Asterix. Huge number of branches and very thin stems were noticed in higher doses of treatments, which indicate that abnormal variants were created at high doses of mutagen [Fig. 3(a)(b)(c)]. Length of shoot per plantlet was the highest (12.47 cm) in the treatment MS+2.0 mg/L of EMS in Asterix. The lowest length (5.32 cm) was recorded in the treatment MS+2.0 mg/L BU with Cardinal. Due to application of chemical mutagen, there was a great variation in shoot formation and shoots per plantlet. In some treatments abnormal shoot development occurred but eventually those shoots died. The treatment MS+4.0 mg/L EMS, MS+2.0 mg/L MMS, MS+4.0 mg/L MMS, MS+2.0 mg/L BU and MS+4.0 mg/L BU had negative effect on shoot development. Some of the experimental materials died at 30 or 45 days of culture in the above treatments which proved toxic effect of chemical mutagens (EMS, MMS and BU) on potato genotypes.

Table 1. Effect of 2, 4-D on callus induction in different potato varieties

Variety	Treatments (mg/L)	Days to Callus Initiation	Size of callus (cm)			Fresh weight (gm)	
			15 days	30 days	45 days	Initial weight 15 days	Final weight 45 days
Diamant	T_1= Normal MS	-	-	-	-	-	-
	T_2=MS+1.0	9.3	0.45	1.10	1.40	0.44	1.90
	T_3=MS+2.0	7.2	0.70	1.50	1.70	0.34	1.90
	T_4=MS+4.0	6.3	0.50	0.98	1.30	1.04	1.39
Cardinal	T_1= Normal MS	-	-	-	-	-	-
	T_2=MS+1.0	8.5	0.72	1.39	1.50	0.99	1.75
	T_3=MS+2.0	7.0	0.72	1.54	1.70	0.64	1.39
	T_4=MS+4.0	5.9	1.02	1.90	2.20	1.04	3.67
Asterix	T_1= Normal MS	-	-	-	-	-	-
	T_2=MS+1.0	10.0	0.61	1.99	2.20	0.98	1.92
	T_3=MS+2.0	8.0	0.70	1.56	2.00	0.77	1.67
	T_4=MS+4.0	7.1	0.79	1.55	1.96	1.04	2.99
	SE±	0.19	0.05	0.30	0.19	0.19	0.09
	LSD	0.58	0.09	0.41	0.21	0.31	0.08
	Level of significance	**	**	**	**	**	**

Table 2. Effect of 2,4-D on plantlet regeneration and variant creation in potato

Variety	Treatments (mg/L)	Shoot per plantlet			Root per plantlet		
		15 days	30 days	45 days	15 days	30 days	45 days
Diamant	T_1= Normal MS	1.0	1.50	2.61	2.0	5.3	7.6
	T_2=MS+1.0	1.3	2.50	3.0	2.6	4.8	6.9
	T_3=MS+2.0	1.9	3.0	7.0 (AB)	3.0	6.7	9.3
	T_4=MS+4.0	2.0	5.0	9.3 (AB)	6.7	10.8	15.6
Cardinal	T_1= Normal MS	1.2	2.3	2.3	1.0	3.0	5.0
	T_2=MS+1.0	1.7	2.9	3.2	2.5	3.1	4.7
	T_3=MS+2.0	3.0	6.8	9.5 (AB)	4.0	4.5	17.90
	T_4=MS+4.0	3.2	9.8 (AB)	12.5 (AB)	3.0	4.0	5.0
Asterix	T_1= Normal MS	1.5	2.8	2.8	2.1	3.5	4.2
	T_2=MS+1.0	2.6	3.5	4.1	3.7	4.9	4.9
	T_3=MS+2.0	3.9	8.7	13.2 (AB)	5.0	7.8	15.3
	T_4=MS+4.0	1.0	- (AB)	- (AB)	-	-	-
	SE±	0.53	0.91	0.87	0.71	0.01	0.05
	LSD	0.18	0.10	0.43	0.26	0.06	0.05
	Level of significance	*	**	**	**	*	**

AB= Abnormal shoot development due to application of chemical mutagen

Table 3. Effect of mutagenic agents on normal and abnormal shoot initiation in potato

Variety	Treatments (mg/L)	Days to shoot initiation	No. of shoots per plantlet			Length of shoot per plantlet (cm)		
			15	30	45	15	30	45
Diamant	T_1= Simple MS	5.1	1.1	2.6	4.2	1.90	5.63	9.3
	T_2=MS+1.0 EMS	4.5	2.3	3.5	4.9	1.70	5.42	10.65
	T_3=MS+2.0 EMS	9.3	1.4	3.1	5.9	1.90	6.41	11.25
	T_4=MS+4.0 EMS	8.8	2.9	-	-	1.80	-	-
	T_5=MS+1.0 MMS	10.7	2.1	4.8	5.6	2.10	7.00	11.46
	T_6=MS+2.0 MMS	8.0	1.0	-	-	2.05	-	-
	T_7=MS+4.0 MMS	9.9	2.1	6.5	13.20	2.00	7.01	12.21
	T_8=MS+1.0 BU	9.6	1.9	4.0	5.10	1.80	6.64	11.12
	T_9=MS+2.0 BU	10.4	2.1	-	-	1.90	-	-
	T_{10}=MS+4.0 BU	7.3	-	-	-	2.10	-	-
Cardinal	T_1= Simple MS	6.1	2.1	3.5	4.9	2.10	5.44	8.6
	T_2=MS+1.0 EMS	7.5	2.7	3.9	4.60	1.70	5.45	10.87
	T_3=MS+2.0 EMS	7.5	2.3	3.2	10.9	1.80	5.50	10.10
	T_4=MS+4.0 EMS	10.1	1.6	9.5	15.8	2.00	4.90	9.37
	T_5=MS+1.0 MMS	8.2	2.6	4.8	5.1	1.60	7.10	12.27
	T_6=MS+2.0 MMS	9.3	1.7	3.5	15.0	2.10	7.05	12.00
	T_7=MS+4.0 MMS	10.4	1.8	13.6	24.8	3.10	6.37	11.27
	T_8=MS+1.0 BU	11.1	1.7	3.9	4.9	1.70	5.22	10.72
	T_9=MS+2.0 BU	12.0	1.0	12.80	14.40	1.80	5.21	5.32
	T_{10}=MS+4.0 BU	13.0	-	-	-	-	-	-
Asterix	T_1= Simple MS	7.2	1.7	3.8	4.40	2.08	7.00	8.2
	T_2=MS+1.0 EMS	7.9	2.9	3.1	4.50	1.90	6.00	11.45
	T_3=MS+2.0 EMS	9.2	2.8	13.0	-	2.10	6.10	12.47
	T_4=MS+4.0 EMS	10.3	2.9	2.6	3.5	2.70	6.20	-
	T_5=MS+1.0 MMS	8.7	1.1	2.2	4.45	1.70	5.35	10.75
	T_6=MS+2.0 MMS	9.5	1.9	5.2	8.0	2.05	5.90	11.52
	T_7=MS+4.0 MMS	9.2	3.1	13.2	19.6	2.91	6.30	10.99
	T_8=MS+1.0 BU	7.9	2.9	5.6	7.3	1.30	4.70	9.00
	T_9=MS+2.0 BU	8.0	3.7	6.8	10.0	1.90	4.80	10.20
	T_{10}=MS+4.0 BU	9.5	2.0	-	-	2.00	-	-
	SE±	0.16	0.76	0.87	0.29	0.71	0.09	0.03
	LSD	1.35	0.99	0.67	0.12	0.28	0.90	0.03
	Level of significance	**	**	**	**	*	*	**

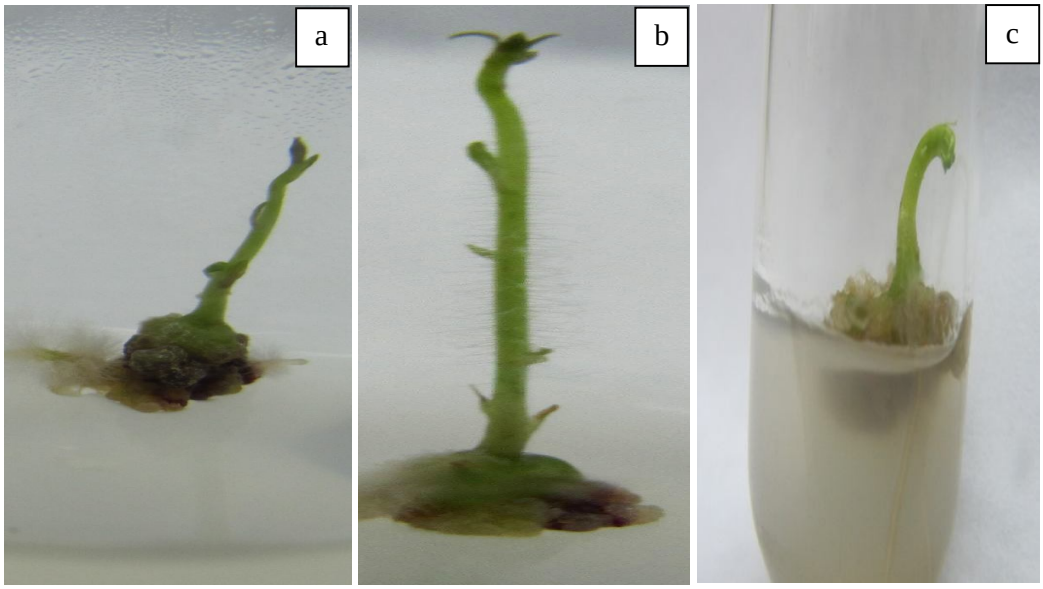

Fig. 1. Healthy plantlet development from callus in the varieties: (a) Cardinal (b) Aterix and (C) Diamant

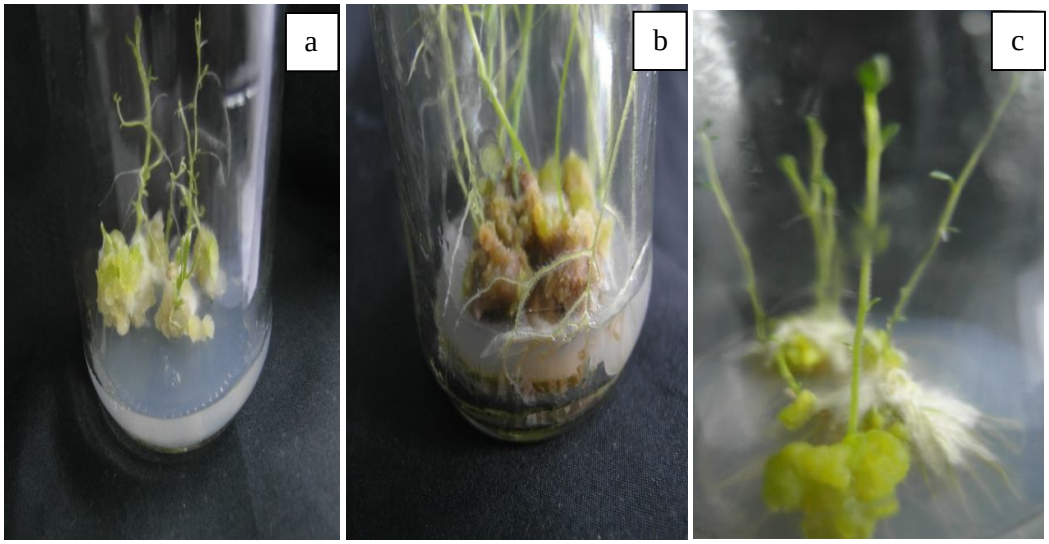

Fig. 2. Abnormal shoot development from callus in (a) Cardinal (b) Asteix and (c) Diamant

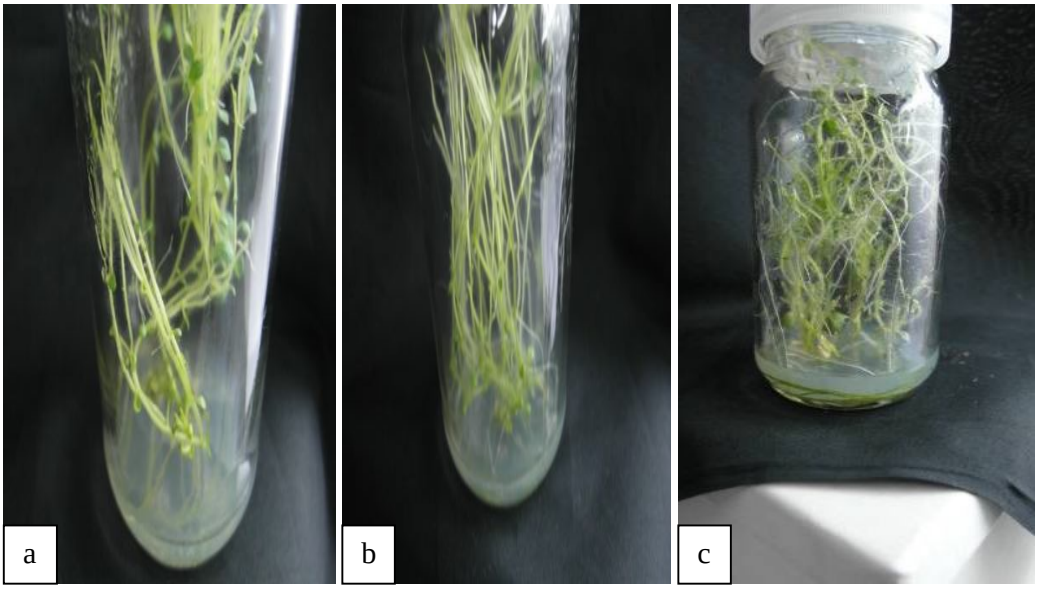

Fig. 3. (a) Abnormal shoot formation due to EMS treatment in Variety-Cardinal
(b) Abnormal shoot formation due to MMS treatment in Variety – Diamant
(c) Abnormal shoot formation due to BU treatment in Variety -Asterix

Fig. 4. Somaclonal variants sown in plastic tray

Table 4. Effect of mutagenic agents on root initiation in potato

Variety	Treatments (mg/L)	Days to root initiation	No. of roots per plantlet		
			15 days	30 days	45 days
Diamant	T_1= Normal MS	9.0	0	5.1	7.2
	T_2=MS+1.0 EMS	10.5	2.1	7.5	18.9
	T_3=MS+2.0 EMS	11.0	3.4	7.6	10.9
	T_4=MS+4.0 EMS	12.3	4.8	-	-
	T_5=MS+1.0 MMS	11.0	0	6.5	8.3
	T_6=MS+2.0 MMS	7.0	1.7	-	-
	T_7=MS+4.0 MMS	8.9	3.2	7.2	11.2
	T_8=MS+1.0 BU	12.5	0	5.5	7.6
	T_9=MS+2.0 BU	10.10	2.5	-	-
	T_{10}=MS+4.0 BU	9.9	-	-	-
Cardinal	T_1= Normal MS	11.1	1.5	4.8	6.9
	T_2=MS+1.0 EMS	9.6	1.1	5.1	9.3
	T_3=MS+2.0 EMS	8.7	3.5	7.7	10.5
	T_4=MS+4.0 EMS	10.5	2.5	6.5	11.6
	T_5=MS+1.0 MMS	14.6	2.1	5.2	7.1
	T_6=MS+2.0 MMS	17.2	2.9	6.5	9.3
	T_7=MS+4.0 MMS	19.2	4.1	6.5	11.7
	T_8=MS+1.0 BU	18.6	1.5	4.2	7.2
	T_9=MS+2.0 BU	10.1	2.1	4.5	9.2
	T_{10}=MS+4.0 BU	11.9	-	-	-
Asterix	T_1= Normal MS	13.0	1.0	3.5	5.5
	T_2=MS+1.0 EMS	9.1	1.8	3.3	7.8
	T_3=MS+2.0 EMS	12.1	3	5.1	8.2
	T_4=MS+4.0 EMS	10.5	2	6.6	-
	T_5=MS+1.0 MMS	6.5	1	4.7	6.1
	T_6=MS+2.0 MMS	8.7	3	5.7	7.1
	T_7=MS+4.0 MMS	9.1	4	5.0	8.0
	T_8=MS+1.0 BU	13.2	2	5.3	7.0
	T_9=MS+2.0 BU	14.8	2	4.4	4.2
	T_{10}=MS+4.0 BU	17.2	3	-	-
	SE±	0.83	0.22	0.63	0.81
	LSD	0.93	0.13	0.32	0.10
	Level of significance	**	**	**	*

Table 5. Survival rate of different regenerated plantlets after transplantation

Acclimatization	Variety	No. of transplanted Plantlets	No. of Plants Survived	Percentage of Survival (%)
In small plastic tray	Asterix	369	66.50	18
	Cardinal	725	152.25	21
	Dimant	847	127.05	15
Sub total		1941	345.80	17.81
In natural field condition under netting	Asterix	66.0	19.8	30
	Cardinal	150.0	49.5	33
	Dimant	125	53.75	43
Sub total		331	123	37.16%

Days to root initiation and number of roots per plantlet are presented in the Table 4. It was observed that the maximum number of days (19.2) was required for root initiation in the treatment MS+4.0 mg/L of MMS Cardinal. Minimum days (6.5) to root initiation was noticed in the treatment MS+1.0 mg/L MMS in the variety Asterix. The highest number (18.9) of roots was found in the treatment MS+1.0 mg/L of EMS in Diamant at 45 days and the lowest (4.2) was in the treatment MS+2.0mg/L BU in Asterix on 45 days after culture of plantlet. *In vitro* mutagenesis and somaclonal variations were studied by Kumar *et al*. (2010). They used physical mutagen (gamma rays) and chemical mutagens *viz*. ethyl methane sulphonate (EMS) and Methyl methane sulphonate (MMS) to induce salt tolerance in a commercial citrus rootstock. The chemical mutagenesis was carried out on 45 and 60 Doc with EMS and MMS treatments at the concentrations of 0.1, 0.2, 0.3 and 10.4%. Results revealed that 0.1% chemical mutagen was the most suitable dose for 45 Doc, whereas 60 Doc did not regenerate after mutagen treatment. The results of the present investigation are similar to those reported by Kumar *et al*. (2010). In addition to this, 4.0 mg/L of EMS, MMS or BU treated cultures died within 45 days after inculcation in the present study.

3.3. Acclimatization efficiency of regenerated variants

Rate of survival of regenerated variants after transplantation are presented in Table 5. Acclimatization efficiency of the regenerated variants was recorded under natural field condition. In the first step, the plantlets were sown in plastic tray for hardening of the culture. A sub total of 1941 variants were transferred to the plastic trays. On an average, only 17.81% of plantlets were able to survive under primary establishment in plastic tray. The well developed variants were transfer to main field and a total of 331 well established variants were sown in the main field. It was noticed that 37.16 % of the variants were able to survive in field condition (Fig. 4).

3.4. Agronomic traits and yields of promising somaclones

Individual care was taken for each of the survived variant. All management practices were done for good crop production. It was observed that most of the variants showed vary poor agronomic performance compared to the check varieties. However, only 19 promising variants were selected for further study. The morphology and yield contributing data of those genotypes are given in Table 6. In respect of plant height and leaf per plant all the somaclonal variant showed lower performance than the check variety. Only three variants *viz.*, SVP 9, SVP 53 and SVP 68 gave larger number of tubers per plant and average weight of tubers. The maximum weight (45.0 g) of mini tubers was found in the check variety Asterix, which was followed by SVP-53 (43.20 g) and check variety Cardinal (40.5). Number of tubers per plant was the highest (21.0) in Cardinal which was followed by Check-2 *viz*. Cardinal (20.0), SVP53 (19.2), SVP-9 (18.0) and SVP 68 (15.20). SVP-53 showed higher yield per plant than the two check varieties, Diamant and Asterix.

Table 6. Major agronomic traits and yield of some promising somaclones under field condition

Sl. No.	Name of Variants	Plant height (cm)	No. of leaves per plant	Stem colour	Morphology and yield contributing traits			Tuber colour	Tuber size
					No. of tubers per plant	Average weight of tuber (g)	Yield per plant (kg)		
1.	SVP-02	29.00	12.0	Green	8.80	10.78	0.09	off white	Medium
2.	SVP-9	37.19	17.0	Light red	**18.00**	12.96	0.21	red	Small
3.	SVP-14	22.18	12.0	Green	8.63	4.94	0.04	Brown	Small
4.	SVP-18	34.74	13.0	Green	9.87	4.50	0.04	off white	Small
5.	SVP-20	22.97	11.0	Light green	12.47	7.83	0.09	off white	Small
6.	SVP-21	41.00	14.0	Green	9.54	3.90	0.03	Brown	Small
7.	SVP-22	23.45	7.0	Green	10.79	5.02	0.05	Brown	Small
8.	SVP-25	32.15	14.0	Green	7.0	2.90	0.02	Brown	Small
9.	SVP-33	20.17	11.0	Green	10.50	5.42	0.05	Brown	Medium
10.	SVP-46	30.00	12.0	Green	10.33	8.34	0.08	Brown	Medium
11.	SVP-53	32.00	11.0	Green	**19.20**	**43.20**	**0.83**	Brown	Medium
12.	SVP-56	32.63	11.0	Green	9.38	5.83	0.05	Brown	Medium
13.	SVP-59	28.93	13.0	Light green	8.44	8.17	0.06	Brown	Small
14.	SVP-68	27.00	9.0	Green	**15.12**	24.18	0.36	Brown	Small
15.	SVP-74	28.35	12.0	Red	9.10	6.21	0.05	Red	Small
16.	SVP-77	19.18	9.0	Light green	10.10	7.36	0.07	Brown	Medium
17.	SVP-86	27.03	11.0	Green	11.85	11.59	0.02	Brown	Medium
18.	SVP-88	35.29	15.0	Green	16.48	8.36	0.41	Brown	Small
19.	SVP-92	24.72	13.0	Red	8.00	3.33	0.02	Red	Small
21.	Cardinal (Ch-1)	52.15	32.0	Green	**21.00**	40.5	**0.85**	Red	Big
22.	Diamant (Ch-2)	49.80	29.00	Green	20.10	35.90	0.72	Brown	Big
23.	Asterix (Ch-3)	57.41	35.00	Light redish	14.5	**45.0**	0.65	Red	Big

4. Conclusions

Somaclonal variation is an enabling technology which can be used in potato improvement. The mutagenic chemicals EMS, MMS, BU and 2, 4-D were used at three concentrations (1.0, 2.0 and 4.0 mg/L) to create somaclonal variations in potato. All the mutagens had negative effect on tissue culture in potato. Cases of death occurred in higher dose. Abnormal shoot development and less leaf formation was observed in the evaluated materials. Survival rates of newly created somaclones were only 17.18% at plastic tray and 37.16% at field condition. One hundred and twenty three somaclonal variants were evaluated under field condition with three check varieties in respect of agronomic traits and yield potentiality. Only one SVP 53 gave higher yield than the two check varieties. Hence, it can be evaluated in subsequent generation for varietal development of potato.

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MOLECULAR DIVERSITY ANALYSIS IN POTATO (*Solanum tuberosum* L.) THROUGH RAPD MARKERS

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ABSTRACT

Random Amplified Polymorphic DNA (RAPD) markers were used to study the molecular diversity of 12 popular potato varieties in Bangladesh. DNA was extracted from tender leaf sample for PCR amplification. The PCR amplified DNA profile was visualized on 2% agarose gel, staining with ethidium bromide. Eight RAPD primers were used to evaluate the genetic diversity of potato varieties. Some total of 36 DNA fragments were amplified and out of them 24 were polymorphic. Those primers generated 61.53% of polymorphic DNA band. The primer OPX 04 produced highest (9) number of DNA band and out of 9 amplicon 6 were polymorphic. Lowest number of amplification was observed in the primer OPA-17 and it was only 3. The highest Nei's genetic distance (0.9701) was noticed between the variety Granola and Provinto. The highest (0.8205) number of genetic identity/similarity was observed between the varieties Cardinal and Diamant. The unweighted pair group method of arithmetic mean (UPGMA) dendrogram based on Nei's genetic distance revealed that the 12 varieties followed into two main clusters. The present finding showed that there was high level of genetic diversity among the varieties which can be used for parental selection in potato breeding program.

Key words: Molecular diversity, RAPD, potato.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is a highly heterogeneous and vegetatively propagated crop. It is one of the important food crops of Bangladesh as well as in many other countries of the word. It produces more calories and protein per unit of land with minimum time than any other field crops (Upadhya, 1995). Because of its high yield potential and food value as compared to rice and wheat, it is cnsidered as a promising candidate crop for feeding the hungry people of the world (Pushkarnath,

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1976). The yield level of potato in Bangladesh is lower than other potato growing countries of the world (BBS, 2010). The use of local seed and traditional varieties are the major constrains of low yield in potato. Development of high yielding varieties having good keeping quality is one of the challenges for potato breeders. Genetic variability has been considered as is prerequisite for crop improvement program. The quantification of genetic diversity made it possible to select diverse parents for successful hybridization program. In recent years, several molecular markers had been used to identify and assess the genetic diversity and phylogenies relationship in plant. The traditional methods based on morphological traits require more time, cost expensive and has large effect on environment. By the development of a wide range of molecular technique, marker assisted breeding is now used to enhance conventional breeding program for crop improvement. Among the different molecular markers RAPD technique (Williams et al. 1990) is reliable, faster and easier for exploiting molecular diversity analysis within and among species. RAPD markers have been widely used for identification of genetic relationship among cultivars (Tosti and Nejri, 2002). Hence, the present investigation was undertaken for molecular diversity analysis of some released potato varieties through RAPD markers and to identify the divergence genotypes for potato improvement program.

MATERIALS AND METHODS

The experiments were carried out at the Department of Biotechnology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka. Twelve popular released potato varieties were collected from Tuber Crops Research Centre (TCRC), Bangladesh Agricultural Research Institute (BARI), Gazipur and used as experimental materials. The potato varieties were Cardinal, Diamant, Granola, Asterix, Sagita, Courrage, Lady Rosetta, Felsina, Multa, Provinto, Petronige and TPS-1.

Seedling raising: Good quality, disease free, healthy tuber (breeder stock) were sown in plastic pots and kept in nets house. All management practices were done for raising quality seedlings from those materials. Fresh leaves were collected at 3-4 leaf stage of plant for isolation of DNA.

Eetraction and quantification of DNA: Total genomic DNA from each variety was isolated by CTAB method with slight modification according to Frey et al., (2004). The extracted DNA was purified by propanol and treated with 10µg/ml RNase A for 20-25 min at 37^{o}C to remove the RNA. The purified DNA was dissolved in TE buffer and quantification of DNA was done through electrophoresis on 1% agarose gel staining by ethidium bromide. The sample DNA was stored at -20^{o}C freezer for further use.

Primer selection and PCR amplification: Seventeen RAPD primers were selected on the basis of previous works to evaluate the molecular polymorphism among the potato varieties. PCR reaction was performed using BIONEER KIT

(korea). The PCR reaction having 20.0 µl mixture containing 3.0 µl sterile deionized water, l0X PCR buffer 4.0 µl, enzyme dilution buffer 4.0 µl, 20 mM $MgCl_2$, 3.0 µl dNTPs (10mM) 1.0 µl top DNA polymerase 0.5 µl, primer 2.5 µl and sample DNA (approx. 40-50 ng) 2.0 µl. The reaction mixture was subjected to the following thermal profile for amplification in a thermocycler : 5 min at 95^{o}C for initial denaturation, followed by 33 cycle of 1.10 min denaturation at 94^{o}C, 1.0 min at annealing and 1.30 min at 72^{o}C for extension. A final extension step was done at 72^{o}C for 7 min. Electrophoresis was done to visualize the PCR amplified product. It was carried out on 2.0% agarose gel and amplified fragments were visualized by staining with ethidium bromide. The amplified bands were scored as present (1) and absent (0) for each primer. The score of bands were pooled to create a single data matrix. These were used to estimate polymorphic loci. Genetic distance and identity were calculated based on Nei's (1972)**.** Phylogenetic tree and dendrogram were established based on an unpaired group method of arithmetic means (UPGMA) using the software POPGENE (Version 1.31) (Yeh et al., 1999).

RESULTS AND DISCUSSION

Molecular diversity and polymorphism studies in 12 potato varieties of Bangladesh was done through RAPD primer. Seventeen RAPD Primers (10-mer) were initially screened on 12 popular potato varieties for their ability to amplify polymoric fragment of DNA. Out of them only eight primers viz. OPA-17, OPG-17, OPJ-13, OPP-12, OPX-01, OPX-04 and OPX-07 showed distinct polymeric DNA profiles. Some total of 39 bands were obtained from these primers with an average of 4.87 bands per primer. Among the amplified product 24 polymorphic DNA bands were observed. The polymorphic DNA fragments ranged from 2-6 in different RAPD oligomer. It was observed that the primer OPX-04 product had highest (9) number of polymorphic DNA band and it was lowest (2) in OPA-17 and OPX-07 primers. The percent of polymorphic DNA fragment was 61.53 under this present investigation (Table 2). The maximum DNA fragment was generated by the primer OPX-04 and it was minimum (3) in OPA-17. The DNA profile of 12 potato varieties using OPX-04, OPX-07 and OPX-17 primers are shown in figure 1 and 2, respectively. The number of polymorphic bands was considered appropriate to assess the genetic divergence of potato genotypes. It might be due to more amount of GC content (60-70%) of the primers used in this study. Fukuoka et al. (1992) observed an increased number of bands with increasing GC content of the primer. The explanation for the correlation between GC content and the number of bands may be the stability of base complementation of A with T. The amplified DNA profiling was scored according to the presence and absence of bands. Absence of bands might be failure of primers to anneal at a binding site in some genotypes due to nucleotide sequence differences or may be insertion or deletions of primer binding site. Rocha et al. (2010) reported on genetic diversity in potato cultivar by RAPD and SSR markers. They notice that, genomic DNA of 16 potato cultivars was amplified with 25 RAPD primers that

generated 92 polymorphic bands. The cultivar identification using RAPD markers is well documented in studies of molecular characterization (Bianchi et al, 2003). Fingerprinting based on RAPD marker type was used for identification and characterization of potato cultivars in North America (Sosinski and Donches, 1996). The genetic identity and genetic distance among the 12 potato varieties are presented in table 2. The Nei's genetic identity was the highest (0.82050) in the varietal pair Cardinal and Diamant and it was the lowest (0.333) in Provinto and Granola. The highest Nei's genetic distance (0.970) was noticed between Granola and Provinto. It was the lowest (0.137) in two different varietal pair viz, (a) Petronige and Provinto (b) Courrage and Provinto, respectively. However, high levels of genetic distance were also noticed in the varietal pairs: Lady Rossetta and Cordage (0.955), Provinto and Lady Rossetta (0.893), Courrage and Granola (0.8910). A dendrogram based on Nei's (1972) genetic distance using unmeasured pair group method of arithmetic mean (UPGMA) was established with 12 popular potato varieties (Figure 3). These varieties segregated into two main clusters. The variety Granola and Sagita were into one cluster and rest of the materials were in cluster-II. The cluster -II was sub-divided into two sub groups. Seven varieties were clustered in one sub-group and three varieties were clustered in second sub-group. Those sub-groups were further segregated in different sub-sub cluster group on the basis of their identity. The results indicated that, low and high level genetic distance exists between the varieties. The variety Granola, Provinto, Lady Rossetta and Courrage showed highest level of genetic diversity which can be used for further potato breeding program. Sawy et al. (2007) reported that, RAPD technique can be successfully applied to determine the genetic fidelity of potato plant. A limited study has been made on genetic divergence in potato either at tetraploid (Gaur et al. 1978 and Sidhu et al., 1981 or at diploid level (Grag 1988). Mondal (2007) reported that an understanding of the nature and magnitude of variability among the genetic stock is of prime importance to the breeders. Hence, it is important to analyze the genetic variability of parental materials. Molecular based analysis of present finding can provide information on actual genetic diversity among the potato cultivars.

CONCLUSION

Information on the genetic diversity allows to assist the parent selection and paving the way to genetic gains. The results of present study revealed the existence of high level of genetic diversity among the studied 12 popular widely grown potato varieties in Bangladesh. These varieties can further be used as parental material for fixation of heterosis in potato improvement program.

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PCR Amplification with RAPD Primer OPX-07

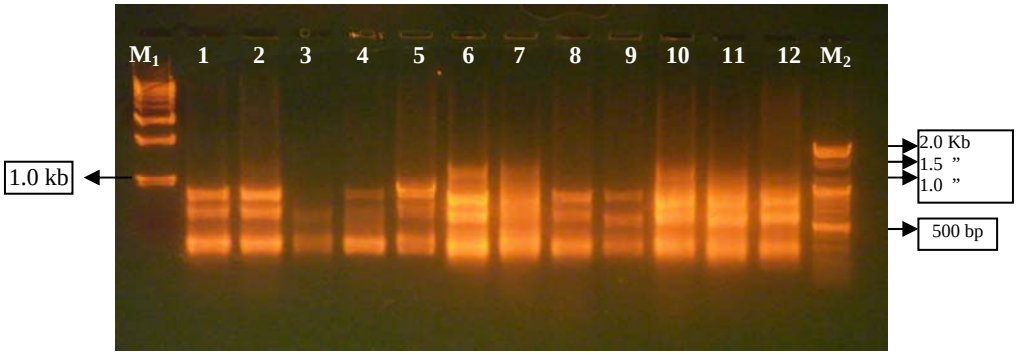

Figure 1: RAPD profile of 12 potato varieties using primer OPX-07. Lane: 1. Cardinal; 2. Diamant; 3. Granola; 4. Lady Rossetta; 5. Sagita; 6. Courrage; 7. Asterix; 8. Felsina; 9. Multa; 10. Provinto; 11. Petronige; 12. T.P.S. 1; M_1= Molecular marker 1kb (B.G. Nei, India) and M_2=100bp (Bioneer, Korea)

PCR Amplification with RAPD Primer OPX-04

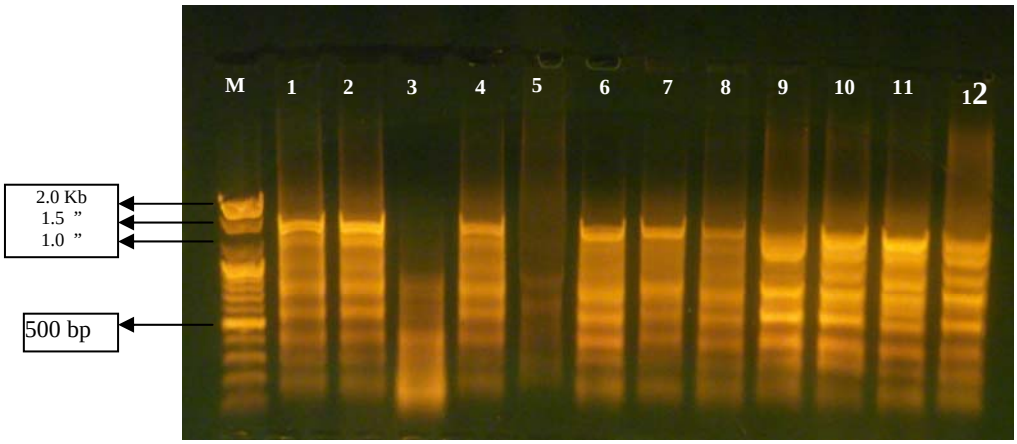

Figure 2: RAPD profile of 12 potato varieties using primer OPX-04. Lane: 1.Cardinal; 2. Diamant; 3. Granola; 4. Lady Rossetta; 5. Sagita; 6. Courrage; 7. Asterix; 8. Felsina;9. Multa; 10. Provinto; 11. Petronige; 12. T.P.S. 1; M= Molecular marker 100bp (Bioneer, Korea)

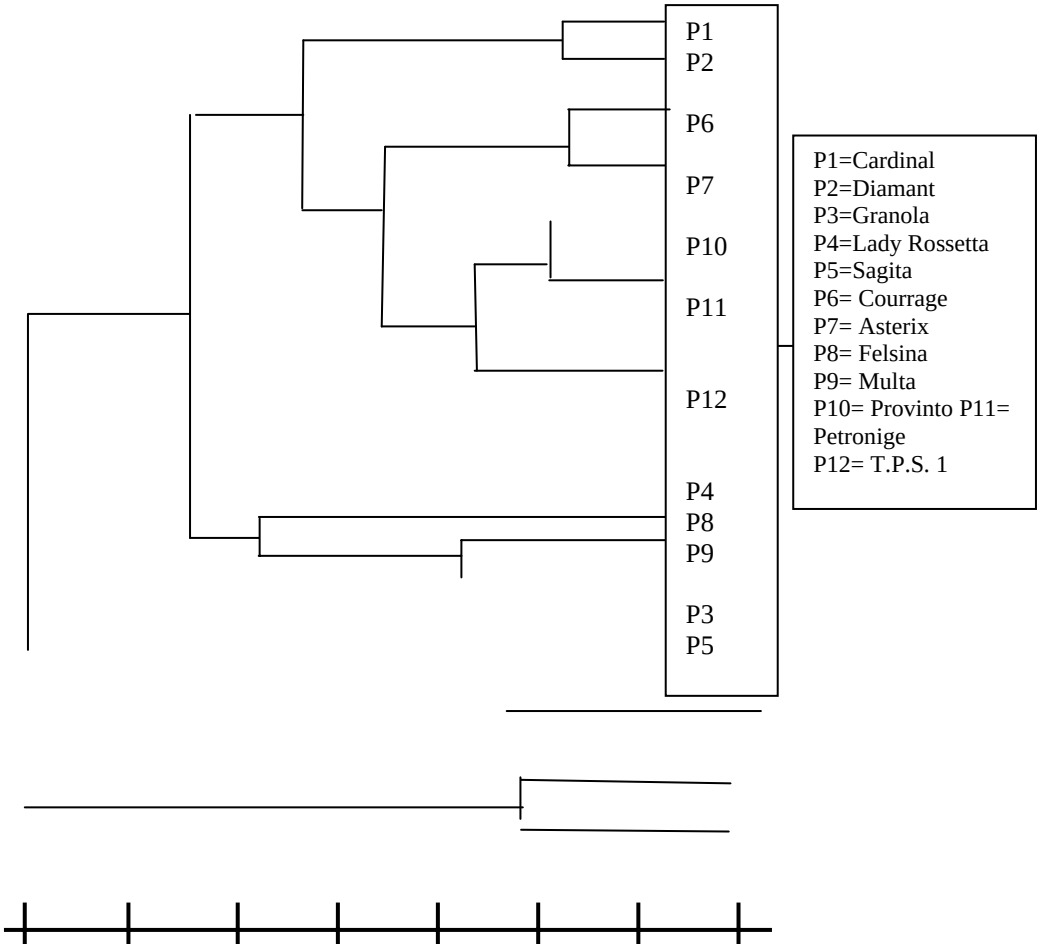

Figure 3: UPGMA dendrogram based on Nei's (1972) genetic distance, between 12 potato varieties according to RAPD analysis.

Table 1: Number and percentage of polymorphic loci obtained in 12 potato varieties

Name of RAPD primer	Sequence of the primer	GC content (%)	No. of bands scored	Size ranges (bp) observed	No. of polymorphic bands	Percentage of polymorphic loci
OPA17	GACCGCTTGT	60	3	278-735	2	66.66
OPG17	ACGACCGACA	60	6	249-1225	3	50.0
OPJ13	CCACACTACC	60	5	295-1491	4	80.0
OPP12	AAGGGCGAGT	60	5	400-1264	3	60.0
OPX01	CTGGGCACGA	70	7	144-934	4	57.14
OPX04	CCGCTACCGA	70	9	198-1579	6	66.66
OPX07	GAGCGAGGCT	70	4	301-1272	2	50.00
Total	-	-	39	-	24	61.53

Table 2: Genetic identity (above diagonal) and genetic distance (below diagonal) values among the twelve potato varieties

	Cardinal	Diamant	Granula	Asterix	Lady Rossetta	Courrage	Sagita	Felsina	Multa	Provinto	Petronige	TPS1
Cardinal	-	0.8205	0.5897	0.6154	0.5641	0.7692	0.6923	0.6410	0.5641	0.7436	0.6667	0.7692
Diamant	0.1978	-	0.6667	0.7436	0.5897	0.6410	0.5641	0.4615	0.5385	0.6667	0.7436	0.6923
Granula	0.5281	0.4055	-	0.6154	0.7692	0.4103	0.4359	0.5385	0.5641	0.3333	0.4615	0.4615
Asterix	0.4855	0.2963	0.4855	-	0.5897	0.5897	0.5641	0.6154	0.6923	0.6154	0.7436	0.6410
L.Rossetta	0.5725	0.5281	0.2624	0.5281	-	0.3846	0.4615	0.6667	0.6923	0.3590	0.4872	0.5385
Courrage	0.2624	0.4447	0.8910	0.5281	0.9555	-	0.8718	0.6154	0.5897	0.8718	0.7436	0.6923
Sagita	0.3677	0.5725	0.8303	0.5725	0.7732	0.1372	-	0.7436	0.6154	0.8462	0.7179	0.6667
Felsina	0.4447	0.7732	0.6190	0.4855	0.4055	0.4855	0.2963	-	0.7692	0.6410	0.5641	0.6667
Multa	0.5725	0.6190	0.5725	0.3677	0.3677	0.5281	0.4855	0.2624	-	0.6667	0.7436	0.6923
Provinto	0.2963	0.4055	0.9701	0.4855	0.8931	0.1372	0.1671	0.4447	0.4055	-	0.8718	0.8205
Petronige	0.4055	0.2963	0.7732	0.2963	0.7191	0.2963	0.3314	0.5725	0.2963	0.1372	-	0.7949
TPS1	0.2624	0.3677	0.7732	0.4447	0.6190	0.3677	0.4055	0.4055	0.3677	0.1978	0.2296	-

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