

## PERFORMANCE AND MOLECULAR ANALYSIS OF POTATO LINES DEVELOPED FROM GAMMA RAYS AND EMS APPLICATIONS

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### ABSTRACT

An experiment was conducted in a greenhouse - research station B - College of Agricultural Engineering Sciences, University of Baghdad, during the fall season of 2018 with the aim of propagating and initially studying the field performance of 18 and 20 potential potato lines derived from Rivera and Arizona cv. after *in vitro* exposure of nodal segments to different dosages of gamma rays (0, 10, 20, and 30 Gray) and EMS (0, 10, 20, and 30 mM). Each control cultivar and their derived lines were independently cultured in plastic bags according to the RCBD, with three replications. The results showed that the highest plant height and number of leaves were obtained from Arizona derived lines which gave 60.11 cm and 25.30 leaves.plant<sup>-1</sup> in lines 207 and 222, respectively when compared with their control that gave 38.11 cm and 13.67 leaves.plant<sup>-1</sup>, respectively. Minitubers diameter, weight, and plant yield were in its highest values in Arizona derived lines 551, 551, and 459 which gave 35.73 mm.minituber<sup>-1</sup>, 33.13 g. minituber<sup>-1</sup>, and 133.8 g.plant<sup>-1</sup>, respectively compared to their control that gave 25.35 mm.minituber, 16.8 g.minituber, and 78.57 g.plant<sup>-1</sup>, respectively. The resulted lines were analyzed at the molecular level utilizing the inter simple sequence repeats (ISSR) markers and revealed that lines 69, 10, 68, 102, and 7 were the much distanced from its derived Rivera cultivar and gave 40.7%, 37.1%, 36.8%, 33.3%, and 30.0%, respectively while lines 551, 261, 474, and 254 were the much genetically distanced from their derived cultivar Arizona with genetic distances of 24.1%, 22.6%, 18.8%, and 17.6%, respectively.

**Key words:** *Solanum tuberosum* L., Mutagenesis, Minitubers, Genetic distance, ISSR  
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الاداء الحقلية والتحليل الجزيئي لخطوط البطاطا المستنبطة من المعاملة باشعة كاما و EMS

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المستخلص

نفذت التجربة في بيت محمي عند المحطة البحثية (B) التابعة لكلية علوم الهندسة الزراعية / جامعة بغداد للموسم الخريفي 2018 لغرض الاكثار و الدراسة الاولية للاداء الحقلية ل 18 و 20 طافر محتمل مشتقة من صنفين من البطاطا هما اريزونا وريفيرا بعد تعريض عقدها مختبريا الى جرعات مختلفة من اشعة كاما (0 و 10 و 20 و 30 غري) والاثيل ميثان سلفونيت (0 و 10 و 20 و 30 ملي مولر). تم زراعة كل صنف مع خطوطه المستنبطة بصورة مستقلة في اكياس بلاستيكية وفق تصميم القطاعات العشوائية الكاملة بثلاث مكررات. اظهرت النتائج ان اعلى ارتفاع للنبات واكثر عدد اوراق تم الحصول عليها من النباتات المشتقة من الصنف اريزونا اذ بلغت 60.11 سم و 25.30 ورقة.نبات<sup>-1</sup> للخطين 207 و 222 بالتتابع عند مقارنتها مع صنف المقارنة التي اعطت 38.11 سم و 13.67 ورقة.نبات<sup>-1</sup> بالتتابع. وظهرت النتائج ايضا ان قطر ووزن الدرينات والحاصل للنبات الواحد كانت عند اعلى قيمها في الخطوط المستنبطة من الصنف اريزونا اذ بلغت 35.73 ملم.درينة<sup>-1</sup> و 34.33 غم.درينة<sup>-1</sup> و 133.8 غم.نبات<sup>-1</sup> للخطوط 551 و 551 و 459 بالتتابع عند مقارنته بصنف المقارنة الذي اعطى 25.35 ملم.درينة<sup>-1</sup> و 17 غم.درينة<sup>-1</sup> و 78.57 غم.نبات<sup>-1</sup> بالتتابع. حلت الخطوط المنتخبة على المستوى الجزيئي باستعمال مؤشرات الدنا البسيطة المترادفة البينية (ISSR) حيث بينت ان الخطوط 69 و 10 و 68 و 102 و 7 كانت الابعد وراثيا عن الصنف ريفيرا التي استنبطت منه اذ اعطت 40.7% و 37.1% و 36.8% و 33.3% و 30% بالتتابع في حين حققت الخطوط 551 و 261 و 474 و 254 اعلى بعد وراثي عن الصنف اريزونا التي اشتقت منه وبلغ 24.1% و 22.6% و 18.8% و 17.6% بالتتابع.

الكلمات المفتاحية: *Solanum tuberosum* L.، التطفير، الدرينات، البعد الوراثي، ISSR

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## INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the four major staple food including wheat, rice and corn and plays a vital role in the international food security. Therefore, improving its field performance against several biotic and abiotic stresses is crucial to elevate crop yield's potential. Mutagenesis aims at the disruption or alternation of genes and can be considered a valuable tool of genetic variations that attract plant breeders. Many phenotypic characterizations that contribute to the domestication and improvement of plant species resulted from human selection for novel traits driven from structural and regulatory genes (19). Simakove et al. (21) tested the effectiveness of multiple backcrossing scheme towards the development of commercial potato varieties. They found high efficiency of tolerant genes introgression in terms of resistance to alternaria, heat, and drought stresses. Many studies have shown the significant effect of induced mutation on plants improvement. Chepkoech et al. (8) were able to induce mutation in potato by exposing potato tubers of three commercial varieties to different dosages of gamma rays. They found that the heritability rates of four agronomical traits including plant height, shoot number, tuber number, and tuber weight were high and gave 62.51%, 52.70%, 64.30%, and 63.43%, respectively which indicates the successful induction of mutation with a high chance for the improved traits to be displayed in the future generation. Ulukapi et al. (25) noticed that high dosage of gamma rays significantly decreased some eggplant growth parameters including plant height (13.58 cm) and shoot fresh weight (1705 g.plant<sup>-1</sup>) compared to their control which gave 20.41 cm and 3.021 g.plant<sup>-1</sup>, respectively. A single potato mutant with high yield potential was produced by Morshad (16) after they subjected potato callus to different types of chemical mutagens. He was able to produce 1941 variants from the applied treatments but only 331 survived under acclimatization and further 37.16% were able to survive the field condition. True potato seeds (TPS) were treated with Ethyl methanesulfonate (EMS) for different durations to induce mutation by Somalraju et al. (23) in order to develop pre-breeding

germplasm. The abnormality percentage of the produced plants was 6.25% ranged from twisted stem, chlorotic and crinkle leaves, purple leaf tip, bushy growth, and slim stem. Although EMS-treated plants tend to produce more tubers per plant but no significant differences were observed between treated and untreated plants in this term. Likewise, no significant differences were noticed in tuber's weight between treated and untreated plants in spite of the higher tuber's weight exhibited in the EMS-treated plants. Khierallah and Jawad (13) Subjected 8 potato varieties to different concentrations of NaCl and found that salinity tolerance significantly differentiated among the investigated genotypes. Taking all together, the aim of this study was to produce sufficient amount of tubers for future propagation in addition to examine some agronomical characteristics of two potato cultivars grown in the greenhouse after their exposure to different levels of gamma rays and EMS.

## MATERIALS AND METHODS

### Lines development

Nodal segments from both cultivars were *in vitro* subjected within four dosages of Gamma rays at 0, 10, 20, and 30 Gray and immersed in 4 concentrations of EMS at 0, 10, 20, and 30 mM for two hours to develop potential lines. The total number of plantlets yielded from the developmental treatments were 800 came from (50 nodes \* 4 dosages \* 2 cultivars \* 2 mutagens). The produced lines were further participated in a series of selection criteria to eliminate the ones that exhibit high mortality rates and growth abnormality.

### Lines acclimatization

The selected lines were acclimatized prior to their transfer to the field. The acclimatization process was held at the Date Palm Research Unit labs / College of Agricultural Engineering Sciences / University of Baghdad for approximately one month. Plantlets were collected from the test tubes, washed thoroughly with tap water to remove the remaining growth medium, and treated with Beltanol fungicide (Fitoagricola.Co) at 1 ml.l<sup>-1</sup>. Afterwards; plantlets were cultured in pre-sterilized peatmoss in 100 ml pots and kept in the incubator at 25°C for two weeks. For the first week, plantlets were watered with half

strength MS salts three times a day and for the second week, plantlets were watered with a solution contained balanced N, P, K, and trace elements twice a day. Subsequently, potted plantlets were transfer to the lath house for further hardening for three weeks prior to their final transfer to the greenhouse.

#### Lines field trial

Acclimatized plantlets were transferred to 15 kg plastic bags contained 1:3 (peatmoss:soil) mixture and grown in the greenhouse for 100 days to obtain sufficient line tubers. The greenhouse location was at the Research Station B - College of Agricultural Engineering Sciences - University of Baghdad. Plants were transferred to the greenhouse in the 5<sup>th</sup> of October, 2018 where all field managements were regularly performed to ensure meeting the crop growing requirements. Potential mutagenic lines and their control cultivars were randomly distributed within each replication in which Arizona and their derived lines accounted for 21 lines while Rivera and their derived lines accounted for 19 lines with 12 plastic bags for each line that represents the experimental unit. The experiment was designed independently for each cultivar and its derived lines according to the Randomized Completely Block Design (RCBD) with three replications and means were compared according to the Least Significant Differences (LSD) test at 5% level of significance. Data collection from a pool sample of 6 randomly selected plants per experimental unit was in regard to: Plant height (cm.plant<sup>-1</sup>), number of stems (stems.plant<sup>-1</sup>), number of leaves (leaves.plant<sup>-1</sup>), shoot dry weight percentage (SDW%), plant yield (g.plant<sup>-1</sup>), minituber average weight (g.minituber<sup>-1</sup>), minitubers number (minitubers.plant<sup>-1</sup>), and minitubers diameter (mm.minituber<sup>-1</sup>).

#### DNA extraction and molecular analysis

Potato genomic DNA was extracted following the protocol established by Chaudhry et al. (6). Five inter-simple sequence repeats (ISSR) primers were obtained following the publications of Bornet et al. (5) and Mahgoub et al. (14). Genetic distances were calculated according to the following equation:

$$\text{Genetic distance} = 1 - ((2nxy) / (nx+ny))$$

where (nxy) represents the number of bands sharing the same molecular size while nx represents the total number of band in exhibition A and ny represents the total number of band in exhibition B. The output data were analyzed using the SPSS program to obtain proximity matrix (18).

#### RESULTS AND DISCUSSIONS:

Three hundred explants from each cultivar (control excluded) were exposed to different dosages of gamma rays and EMS but only 18 lines from Rivera (6%) and 20 lines from Arizona (6.6%) survived the mutagenesis treatments in which their designated names are presented in Table 1.

**Table 1. Designated names of the survived explants from both Rivera (R) and Arizona (A) nodal segments exposed to different dosages of gamma rays and EMS**

Naming	Treatments
(1 - 50)	R , 10 Gy Gamma
(51 - 100)	R , 20 Gy Gamma
(101 - 150)	R , 30 Gy Gamma
(151 - 200)	A , 10 Gy Gamma
(201 - 250)	A , 20 Gy Gamma
(251 - 300)	A , 30 Gy Gamma
(301 - 350)	R , 10 mM EMS
(351 - 400)	R , 20 mM EMS
(401 - 450)	R , 30 mM EMS
(451 - 500)	A , 10 mM EMS
(501 - 550)	A , 20 mM EMS
(551 - 600)	A , 30 mM EMS

#### Vegetative characteristics of generated lines

Results in Table 2 show that 12 and 6 lines derived from Rivera and Arizona cultivars, respectively were significantly higher in plant height when compared with their controls in which their values ranged between 53.33 - 37.89 cm in lines 102 and 373, respectively when compared to Rivera cultivar that gave 30.22. Moreover, the significant values of Arizona derived lines were in between lines 207 and 170 which gave 60.11 - 44.22 cm, respectively when compared with their control that gave 38.11 cm. As for the number of stems, it was observed that two lines derived from Rivera cultivar and designated as 68 and 69 gave significant increases in the number of stems which gave 4.22 and 2.88 stems.plant<sup>-1</sup>, respectively when compared with the control that gave 1.33 stems.plant<sup>-1</sup>. Furthermore, 3 lines derived from Arizona cultivar showed significant increases in the number of stems in which their values were 1.77, 1.67, and 1.67

stems.plant<sup>-1</sup> in lines 222, 254, and 180, respectively when compared with the control that gave 1.22 stems.plant<sup>-1</sup>. The Rivera and Arizona derived lines also showed significant increases in the number of leaves where 9 and 4 lines from Rivera and Arizona, respectively were significantly higher than their control counterparts where the significant values in Rivera derived lines ranged 24.33 – 15.0 leaf.plant<sup>-1</sup> in lines 68 and 360, respectively when compared to their control that gave 11.56 leaf.plant<sup>-1</sup>. The significant values of

Arizona derived lines were between lines 254 and 222 and gave 25.11 - 17.33 leaf.plant<sup>-1</sup>, respectively when compared with their control that gave 13.67 leaf.plant<sup>-1</sup>. Interestingly, we noticed that the highest values of SDW% were obtained from the control genotypes. For Rivera derived lines, no significant differences were observed between line 69 and its control which gave 22.30% while the lowest value was recorded in line 313 that gave 10.43% when compared with the control that gave 24.85%.

**Table 2. Field traits represented by some vegetative characteristics of developed lines as a result of gamma rays and EMS exposure at different levels. SDW%= shoot dry weight percentage**

Rivera derived lines					Arizona derived lines				
Line#	Plant height Cm	Stem #	Leaf #	SDW%	Line#	Plant height Cm	Stem #	Leaf #	SDW%
Gamma developed lines					Gamma developed lines				
4	46.89	1.33	19.44	13.59	153	41.33	1.44	13.89	20.16
7	46.67	1.33	17.33	14.53	154	35.11	1.11	11.78	10.78
9	40.78	1.56	14.22	12.78	162	39.00	1.44	13.44	14.71
10	41.22	1.22	13.67	11.35	165	43.33	1.56	14.11	16.71
68	24.78	4.22	24.33	19.90	166	40.33	1.56	14.00	16.06
69	24.44	2.89	17.00	22.30	170	44.22	1.56	14.22	23.16
77	40.67	1.33	14.44	12.38	180	43.67	1.67	15.00	22.89
102	53.33	1.67	18.44	17.60	205	54.44	1.33	22.33	18.22
114	27.78	1.56	11.33	17.20	207	60.11	1.22	16.22	23.06
115	23.56	1.89	13.33	10.68	209	39.44	1.22	13.11	14.08
EMS developed lines					EMS developed lines				
305	29.00	1.44	10.11	18.10	222	39.11	1.78	17.33	25.30
313	47.00	1.00	15.56	10.43	254	45.33	1.67	25.11	23.11
321	52.78	1.33	17.67	13.66	257	49.11	1.33	14.56	15.01
334	30.67	1.56	12.00	18.31	261	42.44	1.44	21.56	25.24
349	45.22	1.11	13.78	13.81	262	34.33	1.56	13.11	20.14
360	38.56	1.11	15.00	15.39	270	42.33	1.33	13.67	23.15
373	37.89	1.67	15.44	14.03	EMS developed lines				
423	39.89	1.22	13.89	14.24	459	48.22	1.22	14.00	23.95
R	30.22	1.33	11.56	24.85	463	37.44	1.33	13.44	23.05
LSD <sub>0.05</sub>	6.52	0.64	3.21	3.17	474	37.56	1.33	13.11	24.96
					551	40.00	1.33	13.11	25.20
					A	38.11	1.22	13.67	26.57
					LSD <sub>0.05</sub>	5.85	0.42	3.24	3.78

Nevertheless, 11 Arizona derived lines did not exhibit significant differences in this term. These results were in agreement with the findings of other researchers (1, 9, 17) that mutagenesis treatment of potatoes showed a significant effect in increasing plant height, number of stems, and number of shoots of produced lines. The exhibited reduction in the SDW% can be attributed to elevated water retention in the generated lines compared to their control as a result of some morphological and physiological modifications in mutants body including changes in the cuticle barrier layer (7) or controlling the stomatal conductance (15) in addition to reducing the rate of transpiration (26).

#### Lines Yield characteristics

The results in Table 3 show that, in the term of minituber weight, 5 Rivera derived lines did not significantly differ from the control and gave a values fallen between 17.18 - 14.60 g.minituber<sup>-1</sup> in lines 360 and 423, respectively compared to the control that gave 17.21 g.minituber<sup>-1</sup>. Nevertheless, 10 Arizona derived lines were significantly higher in minituber weight with the values between 33.13 - 21.80 g.minituber<sup>-1</sup> in lines 551 and 154 when compared with the control genotype that gave 16.80 g.minituber<sup>-1</sup>. The highest number of minitubers in Rivera derived lines was obtained from line 68 which gave 5.11 minituber.plant<sup>-1</sup> though it did not significantly differ from the control that gave 4.88

minituber.plant<sup>-1</sup>; however, most lines were significantly lower than the control in this term. On the other hand, all Arizona derived lines were lower than the control in the term of the number of minitubers although 3 lines

recorded non-significant differences with the values of 4.28, 4.22, and 4.22 minituber.plant<sup>-1</sup> in lines 153, 207, and 474, respectively compared to the control that gave 4.67 minituber.plant<sup>-1</sup>.

**Table 3. Field traits represented by some yield characteristics of developed lines as a result of gamma rays and EMS exposure at different levels**

Rivera derived lines					Arizona derived lines				
Line#	Minituber weight G	Minituber #.plant <sup>-1</sup>	Minituber diameter.plant <sup>-1</sup> Mm	Yield.plant <sup>-1</sup> G	Line#	Minituber weight G	Minituber #.plant <sup>-1</sup>	Minituber diameter.plant <sup>-1</sup> Mm	Yield.plant <sup>-1</sup> G
Gamma developed lines					Gamma developed lines				
4	14.65	3.22	23.55	47.25	153	15.81	4.28	26.03	67.65
7	12.07	3.78	22.08	45.50	154	21.80	2.28	22.31	29.08
9	13.35	3.00	22.23	39.69	162	15.61	2.50	22.81	38.92
10	10.09	3.06	21.27	30.71	165	18.65	3.06	25.03	57.00
68	12.99	5.11	22.79	66.34	166	18.68	2.78	25.84	51.84
69	11.04	4.89	20.52	53.51	170	22.96	4.00	27.78	91.78
77	10.21	3.94	20.63	39.92	180	22.61	3.22	27.31	72.97
102	13.31	4.00	23.59	52.74	205	19.34	2.83	24.60	54.73
114	15.53	3.22	23.34	49.91	207	30.08	4.22	33.98	127.0
115	8.19	2.56	20.16	20.72	209	10.95	2.67	22.93	28.06
EMS developed lines					EMS developed lines				
305	13.09	3.28	23.04	42.91	222	22.83	3.83	27.86	87.32
313	8.55	2.28	18.33	19.32	254	24.17	3.78	27.40	91.53
321	9.65	3.88	22.05	36.95	257	16.16	2.33	24.07	37.64
334	16.54	3.33	24.64	55.05	261	32.89	3.83	33.73	125.9
349	8.96	3.67	20.43	32.99	262	16.36	3.11	23.77	50.41
360	17.18	2.61	23.00	44.89	270	23.35	3.00	30.41	70.07
373	12.40	2.78	21.44	33.23	EMS developed lines				
423	14.60	2.56	23.51	36.70	459	32.55	4.11	32.90	133.8
R	17.21	4.88	25.65	82.91	463	17.66	3.67	26.95	64.76
					474	19.36	4.22	27.37	81.49
					551	33.13	3.17	35.73	104.2
					A	16.80	4.67	25.35	78.57
LSD <sub>0.05</sub>	2.98	0.70	1.69	11.26	LSD <sub>0.05</sub>	2.70	0.52	1.44	13.04

The results in Table 3 show that most of the Rivera derived lines were significantly lower in minituber diameter with an exception in line 334 that gave 24.64 mm.minituber<sup>-1</sup> and did not significantly differ when compared with the control which gave 25.65 mm.minituber<sup>-1</sup>. On the contrast, 10 Arizona derived lines were significantly higher than the control in the term of minituber diameter in which the significant values were between 35.73 - 26.95 minituber.plant<sup>-1</sup> in lines 551 and 463, respectively when compared to the control that gave 25.35 mm.minituber<sup>-1</sup>. As for the plant yield, the results were correlated with the previous yield traits and showed that all Rivera derived lines were significantly lower than the control with the lowest value of 19.32 g.plant<sup>-1</sup> in line 313 when compared with the control that gave 82.91 g.plant<sup>-1</sup>. However, 5 Arizona derived lines were significantly higher in the term of plant yield with the significant values ranged 133.8 - 91.78 g.plant<sup>-1</sup> in lines 459 and 170, respectively when compared with the

control that gave 78.57 g.plant<sup>-1</sup>. Similar findings were reported by (2, 11) when they found that the genotypes used in their experiments had a significant effect on the yield traits which proven that the selection of suitable genotypes for mutation studies is crucial for plant improvement programs where some genotypes are more applicable for induced mutation comparing with others. Gamma rays have a biological effect on the plant cell which based on its interaction with particular molecules especially water and in result produce free radicals (12). These produced radicals can damage or modify the physical and molecular structure of the cell and therefore differentially affect plant morphology, physiology, anatomy, and biochemistry depending on the used dosages of mutagens (3). According to the morphological and yield characteristics obtained in our results, it was noticed that the two cultivars used in our study varied in their response to mutagenesis applications which are

in consistence with many other previous reports (2, 4, 10).

#### Molecular analysis of developed lines

The highest genetic distances between Rivera derived lines and their control were observed in lines 69, 10, 68, 102, and 7 as presented in Table 4 and gave 40.7%, 37.1%, 36.8%, 33.3%, and 30.0%, respectively while the lowest was recorded between Rivera cultivar and line 4 which gave 18.8%. Moreover, the results in Table 5 show that the highest genetic distances between Arizona derived lines and their control were in lines 551, 261, 474, and 254 which gave 24.1%, 22.6%, 18.8%, and 17.6%, respectively while the lowest was noticed in line 153 and gave 5.9%. The ISSR markers are very useful molecular tool for

genetic discrimination among mutants. It was considered very reliable, relatively inexpensive, and gave similar results when repeated (20, 24). The results were similar to the findings of Sobieh et al. (22) when they applied different gamma ray dosages on potato and found that the highest genetic distance was 39.9% while the lowest was 3.3%. We noticed that genotypes can play a vital role in responding to different types of mutagens where Rivera showed to be more genetically affected than Arizona in this manner. This was also reported by Bado et al. (4) when they irradiated 6 potato genotypes with gamma rays and found that genotypes had a significant effect on the irradiation sensitivity.

**Table 4. Genetic distance values among Rivera cultivar and its derivative lines resulted from gamma rays and EMS exposure at Different levels. R=Rivera**

Lines	R	4	114	68	69	7	102	77	10	9	305	349	334	321	373
R	.000	.188	.257	.368	.407	.300	.333	.250	.371	.333	.257	.257	.250	.250	.273
4		.000	.135	.200	.241	.190	.200	.059	.189	.200	.135	.135	.118	.118	.143
114			.000	.116	.313	.111	.211	.189	.200	.105	.200	.150	.189	.189	.211
68				.000	.371	.125	.220	.150	.116	.171	.256	.209	.200	.200	.220
69					.000	.351	.200	.241	.250	.267	.250	.250	.172	.172	.200
7						.000	.209	.190	.156	.209	.156	.156	.190	.190	.209
102							.000	.143	.105	.167	.158	.158	.086	.086	.111
77								.000	.135	.200	.135	.135	.059	.059	.086
10									.000	.263	.200	.150	.135	.135	.158
9										.000	.263	.263	.200	.200	.222
305											.000	.050	.081	.081	.053
349												.000	.081	.081	.053
334													.000	.000	.029
321														.000	.029
373															.000

**Table 5. Genetic distance values among Arizona cultivar and its derivative lines resulted from gamma rays and EMS exposure at Different levels. A=Arizona**

Lines	A	153	165	180	222	270	207	254	170	262	261	459	474	463	551
A	.000	.059	.091	.152	.158	.152	.161	.176	.125	.125	.226	.125	.188	.152	.241
153		.000	.086	.143	.200	.200	.212	.222	.176	.176	.273	.176	.235	.200	.290
165			.000	.176	.179	.176	.125	.143	.152	.091	.250	.152	.212	.176	.267
180				.000	.179	.176	.313	.200	.273	.273	.313	.273	.273	.294	.333
222					.000	.128	.243	.150	.211	.158	.243	.158	.211	.179	.314
270						.000	.125	.086	.152	.212	.250	.212	.273	.235	.267
207							.000	.091	.097	.161	.200	.226	.290	.250	.214
254								.000	.118	.176	.212	.235	.294	.257	.226
170									.000	.125	.097	.125	.188	.152	.103
262										.000	.161	.063	.125	.091	.241
261											.000	.097	.097	.125	.143
459												.000	.063	.030	.172
474													.000	.030	.241
463														.000	.200
551															.000

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