Somaclonal Variation Causes Changes in the Inter-Relationships between Traits in Hexaploid Triticale

P. J. KALTSIKES and P. J. BEBELI

*Department of Plant Breeding and Biometry
Agricultural University of Athens, Athens, Greece*

Two pairs of near isogenic lines of hexaploid triticale ($\times$ *Triticosecale* Wittmack) differing by the presence or absence of telomeric heterochromatin on rye chromosome arms 7RL (cv. Drira) and 6RS (cv. Rosner) were used in this study. Immature embryos of these lines were cultured *in vitro* and plants regenerated from them. The aim of the study was to ascertain whether these processes were associated with changes in the correlations between agronomic traits of the regenerated plants. Several hundreds of R_2 regenerated plants of each line and of their sexually propagated corresponding controls were planted in the field in a completely randomized design with all plants individually randomized. Twelve agronomic traits were studied including heading date, tillering, plant height, yield and its components and spike characteristics. Correlation coefficients between all possible pairs of traits were calculated separately in the regenerants and the controls of each line.

It was found that from one fourth to almost half of the correlation coefficients were statistically significantly different in the regenerants as compared to their corresponding values in the controls. The number of coefficients changing significantly in magnitude was different in the two lines of each cultivar and could not be associated with the presence or absence of telomeric heterochromatin. The traits affected the most were number of days to heading, plant height, plant weight and spike length. Most correlation coefficients became smaller in magnitude in the regenerants.

KEY WORDS: *Triticosecale*, correlations, agronomic traits, somaclonal variation, *in vitro* culture

Introduction

In hexaploid triticale ($\times$ *Triticosecale* Wittmack), telomeric heterochromatin on rye chromosome arms 7RL and 6RS has been shown to be associated with changes in the mean expression of agronomic traits (BEBELI and KALTSIKES unpublished; BENNETT and GUSTAFSON 1982) which are accompanied by changes in their inter-relationships (BEBELI and KALTSIKES unpublished). The induction of somaclonal variation in the same materials has also been shown to be accompanied by changes in the mean expression of agronomic traits (BEBELI *et al.* unpublished; BEBELI and KALTSIKES 1991) relative to un-treated, sexually propagated controls. It would be of interest to examine whether these changes in the regenerants are also associated with changes in the correlations between the various agronomic traits relative to the controls.

In this paper, which describes the results of one of a series of experiments designed to investigate the possible effects of telomeric heterochromatin on the outcome of *in vitro* culture and plant regeneration in hexaploid triticale, we report that considerable changes take place in the correlations between agronomic traits as a result of *in vitro* culture of immature embryos and plant regeneration.

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Materials and Methods

The materials used have been described by MERKER (1976) and ROUPAKIAS and KALTSIKES (1977). They consist of two sets of near isogenic lines of hexaploid triticale ($\times$ *Triticosecale* Wittmack) differing by the presence (++) or absence (--) of telomeric heterochromatin on chromosome arms 7RL (cv. Drira) and 6RS (cv. Rosner). Immature embryos of these lines were cultured *in vitro* and plants regenerated from them according to the procedure described by BEBELI *et al.* (1988). Seeds from 50 different R_2 families per line were germinated in Jiffy 7 tablets and then planted in the field at distances of 20 cm from row to row and from plant to plant. The plants of each line were individually randomized in the field. The same was done for the seeds of the controls which were obtained from plants that were never cultured *in vitro* and regenerated. The seeds used for this experiment originated from plants which were treated identically in the previous generation. The whole experiment was repeated twice. The number of plants which survived to maturity were 525, 424, 509, and 574 for the regenerated plants of lines 7RL++, 7RL--, 6RS++ and 6RS--, respectively. The corresponding numbers of plants for the controls were 106, 78, 81 and 81.

The following twelve agronomic traits were studied number of days to heading (from January 1), number of tillers per plant, plant height, plant weight, spike weight, seed weight, number of spikes per plant, spike length, number of spikelets per spike, number of seeds per spike and harvest index (more details are given in BEBELI *et al.* 1992).

Simple correlation coefficients were calculated between all possible pairs of the traits. Correlation coefficients were tested for differences according to the method described by STEEL and TORRIE (1960). The significance of the differences between the correlation coefficients of the control and regenerated populations for each pair of traits and lines is at least at $p = 0.05$.

Results

It is evident from Table 1 that the relationship between plant weight and the other traits studied changed significantly in the regenerants relative to the controls of line 7RL++. In all, 16 of the correlation coefficients between traits were significantly smaller in the regenerants than in the controls.

More than twice the number of correlation coefficients in the regenerants of line 7RL-- relative to its sister line (7RL++) were significantly different from those in the corresponding controls (Table 2). Almost two thirds of the coefficients were lower in the regenerants. In general in those traits where the correlation coefficients changed all coefficients changed in the same direction as to magnitude.

Of the 32 coefficients which were significantly different in the regenerants from their counterparts in the controls, 20 were lower in magnitude in line 6RS++ (Table 3). In common with the other lines, when a relationship changed in magnitude for a particular trait the change was in the same direction for all its correlation coefficients with the other traits. The relationship of heading date with the remaining traits was affected considerably, with its correlation coefficients becoming lower in the regenerants in all cases but one. The oppo-

Table 1. Comparisons of correlation coefficients between traits in regenerated (R) and control (C) populations of Drira 7RL + +

Trait		tillers/ plant	plant height	plant weight	spike weight	seed weight	spikes/ plant	spike length	spike- lets per plant	seeds/ spike	Harvest index	spike density
Heading date	R	-0.7264	-.3023	-.2638	-.6499	-.6346	-.6244	-.4874	-.3891	-.5785	-.4569	.4581
	C	-0.6554	-.3050	<u>-.6215</u>	-.6472	-.6464	-.5884	-.6064	-.5224	-.4705	-.3880	.4061
Tillers/plant	R		.3259	.2422	.6538	.6184	.6994	.3867	.3194	.4893	.2705	-.3426
	C		.2363	.6106	.5422	.5209	.6611	.4487	.4102	.3551	.1233	-.2586
Plant height	R			.1974	.4377	.4310	.4822	.3661	.3526	.4644	.2250	-.2530
	C			.5026	.4477	.4448	.5250	.3539	.3659	.4910	.0989	-.1606
Plant weight	R				.2967	.5001	.4185	.2644	.2131	.3060	.1211	-.2267
	C				<u>.9427</u>	<u>.9189</u>	<u>.8584</u>	<u>.6902</u>	<u>.5168</u>	<u>.7362</u>	<u>.2956</u>	<u>-.5414</u>
Spike weight	R					.9561	.8550	.5277	.4159	.7488	.5604	-.4892
	C					<u>.9875</u>	.8164	<u>.7029</u>	.5262	.8152	.5214	-.5493
Seed weight	R						.8525	.5161	.4164	.7552	.5571	-.4643
	C						<u>.7845</u>	<u>.6716</u>	.5119	.8127	.5285	-.5151
Spikes/plant	R							.5018	.4164	.6908	.4358	-.4518
	C							<u>.6471</u>	.5454	.6680	.3391	-.4362
Spike length	R								.7396	.4957	.3753	-.7226
	C								<u>.8649</u>	<u>.7151</u>	<u>.5505</u>	<u>-.6932</u>
Spikelets/spike	R									.4801	.3632	-.1443
	C									.6087	.5093	-.2489
Seed/spike	R										.5036	-.3943
	C										.5865	-.4650
Harvest index	R											-.2955
	C											-.3559

Coefficients underlined are statistically different from their counterpart N=525 for the regenerants and 106 for the controls

site was true in its sister line (6RS--).

Twenty five of the correlation coefficients in line 6RS-- were significantly different in the regenerants in comparison with their counterparts in the controls (Table 4). Of these 15 were smaller in size in the regenerants. All changes in the relationship of heading date with the other traits were in the same direction i.e. the coefficients became greater in value. In the other lines where the relationship of heading date with the other traits was affected in the regenerants the correlation coefficients became generally smaller in magnitude.

Discussion

Somaclonal variation has been shown to affect the expression of many traits in plants including morphological traits, resistance to biotic and abiotic stresses, fruit quality characteristics and agronomic traits (KARP 1991). Some of these traits and in particular the agronomic

Table 2. Comparisons of correlation coefficients between traits in regenerated (R) and control (C) plants of line 7RL--

Trait		tillers/ plant	plant height	plant weight	spike weight	seed weight	spikes/ plant	spike length	spike- lets per spike	seeds/ spike	Harvest index	spike density
Heading date	R	-.3418	-.1381	-.1215	-.2946	-.2846	-.2784	-.0838	.0320	-.1587	-.0787	.0376
	C	<u>-.6322</u>	.0313	-.2168	-.5868	-.5969	-.5184	-.5777	-.4907	-.5687	-.5769	.2656
Tillers/plant	R		.2881	.2692	.6448	.6334	.6634	.3969	.0346	.4347	.2943	-.2318
	C		.1521	.2193	<u>.4998</u>	<u>.4713</u>	.5484	.2870	<u>.3387</u>	.3637	.2312	<u>.0075</u>
Plant height	R			.1881	.5233	.5107	.4962	.5522	.1588	.6149	.1911	-.2174
	C			.1221	<u>.3216</u>	.2794	.2698	<u>.1005</u>	.1690	<u>.3604</u>	.1987	<u>.0357</u>
Plant weight	R				.1386	.4805	.4149	.2685	.0539	.3029	.1176	-.1147
	C				.0499	.4883	.4680	.2736	.1620	.3251	.1826	-.2091
Spike weight	R					.9062	.8526	.5940	.0987	.7158	.4218	-.2923
	C					.8791	<u>.7342</u>	.7278	<u>.6145</u>	<u>.8341</u>	<u>.7001</u>	-.3778
Seed weight	R						.8539	.5935	.0979	.7441	.4516	-.2915
	C						.8017	<u>.7631</u>	<u>.6133</u>	<u>.8708</u>	<u>.7193</u>	-.4254
Spikes/plant	R							.5810	.0876	.6150	.3659	-.2949
	C							.6733	<u>.5515</u>	.7166	.5252	-.3667
Spike length	R								.3803	.7528	.3924	-.2840
	C								<u>.8012</u>	.7476	<u>.7139</u>	-.5740
Spikelets/spike	R									.2440	.1263	.7741
	C									<u>.6508</u>	<u>.5691</u>	<u>.0245</u>
Seed/spike	R										.4909	-.2607
	C										<u>.7694</u>	-.3705
Harvest index	R											-.1547
	C											<u>-.4284</u>

Coefficients underlined are statistically different from their counterpart N=424 for the regenerants and 78 for the controls

traits cannot be considered as independent of one another. Usually one trait follows the other ontogenetically and it is reasonable to expect that if the expression of one trait is altered by any agent then those that follow it in ontogenesis might also be affected.

The relationships between the traits can be examined through their correlation coefficients. These coefficients do not imply cause and effect relationships but are indicative of a degree of inter-dependence which is the result of the fact that they all are part of the organism—the plant.

In the present study almost the same number of correlation coefficients changed significantly in each cultivar but the distribution of the changed correlation coefficients was different in the two sister lines of each cultivar. Thus in Drira most correlations changed significantly in the line lacking telomeric heterochromatin while the opposite was true for Rosner.

Table 3. comparisons of correlation coefficients between traits in regenerated (R) and control (C) plants
Line 6RS+ +

Trait		tillers/ plant	plant height	plant weight	spike weight	seed weight	spikes/ plant	spike length	spike- lets per spike	seeds/ spike	Harvest index	spike density
Heading date	R	-.6635	-.2685	-.4137	-.3939	-.3014	-.4840	-.3921	-.3202	-.2486	-.2528	.0909
	C	-.6630	-.0597	-.2873	-.6210	-.6277	-.6093	-.6353	-.5189	-.5603	-.5075	.4970
Tillers/plant	R		.1820	.4761	.4029	.3319	.5323	.2516	.2366	.1682	.0924	-.0187
	C		.1791	.2045	.6107	.5423	.6305	.3286	.3065	.3822	.2282	-.1757
Plant height	R			.4910	.4959	.4605	.4477	.3910	.2814	.4693	.3356	-.1716
	C			.0156	.3571	.2488	.3014	.0552	.0470	.3665	.0988	-.0948
Plant weight	R				.8659	.8064	.7608	.4819	.3391	.5723	.2389	-.2452
	C				.0485	.5073	.3978	.3396	.2350	.3226	.1947	-.3077
Spike weight	R					.9122	.7674	.5355	.3733	.6729	.5718	-.2840
	C					.8567	.7459	.5361	.4214	.7454	.5628	-.4850
Seed weight	R						.6719	.4665	.3228	.6648	.5344	-.2516
	C						.7470	.6135	.4602	.7878	.6060	-.5673
Spikes/plant	R							.5228	.3614	.4852	.3752	-.2691
	C							.6181	.5163	.6231	.4175	-.4923
Spike length	R								.8637	.4903	.5093	-.2091
	C								.9230	.7014	.4782	-.6296
Spikelets/spike	R									.4011	.4149	.2893
	C									.6303	.3935	-.2906
Seed/spike	R										.5759	-.1347
	C										.6368	-.4822
Harvest index	R											-.1628
	C											-.3825

Coefficients underlined are statistically different from their counterpart N=509 for the regenerants and 81 for the controls

Number of days to heading was the trait in which correlations with the other traits were significantly affected the most, particularly so in the two lines which lacked telomeric heterochromatin (7RL-- and 6RS--). In contrast, most significant changes in the correlation coefficients of plant weight with the other traits were observed in regenerants of the lines possessing telomeric heterochromatin.

No clear relationship could be established, however, between the presence or absence of telomeric heterochromatin and changes in inter-trait relationships. This result was somewhat unexpected in that in this series of experiments as well as in others (KALTSIKES and BEBELI 1992; KALTSIKES *et al.* 1984; THOMAS *et al.* 1980) heterochromatin has been shown to affect trait expression and inter-relationships. It might be argued that telomeric heterochromatin has no effect on trait correlations in the regenerated plants. This finding could constitute yet another report of the lack of such an effect in addition to those reported by Gupta and

Table 4. Comparisons of correlation coefficients between traits in regenerated (R) and control (C) plants of Line 6RS--

Trait		tillers/ plant	plant height	plant weight	spike weight	seed weight	spikes/ plant	spike length	spike- lets per spike	seeds/ spike	Harvest index	spike density
Heading date	R	-.6307	-.3293	-.5373	-.5394	-.4985	-.5736	-.2956	-.3853	-.4526	-.4516	.0890
	C	-.1462	-.1185	-.1499	-.1561	-.1430	-.1832	-.1806	-.1600	-.1360	-.1237	-.0098
Tillers/plant	R		.2054	.6232	.6192	.5442	.7421	.1970	.2647	.3194	.2863	-.0769
	C		.2775	.6603	.6874	.6660	.7341	.3832	.3457	.4417	.3711	.0616
Plant height	R			.4216	.4252	.4100	.3732	.2999	.3977	.5661	.4779	-.0638
	C			.5351	.5101	.4914	.4532	.6267	<u>.6192</u>	.6557	.4915	.2064
Plant weight	R				.9570	.9168	.8957	.3294	.3669	.6740	.4312	-.2288
	C				.9667	<u>.9512</u>	.9213	<u>.6485</u>	.5062	.7345	.4642	-.0362
Spike weight	R					.9771	.8720	.3263	.3719	.7272	.5993	-.2137
	C					<u>.9904</u>	.9216	<u>.6266</u>	.4751	.7528	.6087	-.0613
Seed weight	R						.7906	.3005	.3404	.7403	.6110	-.1976
	C						<u>.8862</u>	<u>.5940</u>	.4526	.7587	.6008	-.0562
Spikes/plant	R							.3186	.3908	.5560	.4463	-.1884
	C							<u>.5977</u>	.4561	.6340	.4932	-.0332
Spike length	R								.4477	.3832	.3064	-.5201
	C								<u>.8299</u>	.7431	.5133	<u>.0552</u>
Spikelets/spike	R									.4615	.4059	.3501
	C									<u>.6154</u>	.5233	<u>.5976</u>
Seed/spike	R										.6942	-.1751
	C										.7220	.0167
Harvest index	R											-.1019
	C											<u>.1936</u>

Coefficients underlined are statistically different from their counterpart N=574 for the regenerants and 81 for the controls

Reddy (1991). We feel otherwise and intend to take up this issue elsewhere.

What became clear from the results of this experiment is that the induction of somaclonal variation was accompanied by many changes in the correlation coefficients between traits. The changes in the magnitude and/or sign of the correlation coefficients observed in the regenerated plants could be useful in cases where a negative correlation between two important agronomic traits must be broken. Thus *in vitro* culture and plant regeneration can be added to hybridization and mutagenesis as a major means at the disposal of the breeder for breaking undesirable trait correlations.

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ソマクローナル変異が六倍体ライコムギの形質間相互関係に変化を起こす

P. J. KALTSIKES · P. J. BEBELI

(アテネ農科大学 植物育種および生物測定学部, アテネ市 ギリシャ)

六倍体ライコムギ ($\times$ *Triticosecale* Wittmack) の準同質系統 2 対を本研究に用いたが、それらはライコムギ染色体腕 7 RL (品種, Driva) と 6 RL (品種, Rosner) の端部異質染色質の存否で異なっている。これら 4 系統の未熟胚を試験管内で培養し、植物体を再分化させた。研究目的はこれらの過程が再分化個体の農業質間関係の変化と関係するかどうかを確かめることであった。各系統の再分化植物 R_2 および種子増殖した対照植物のそれぞれ数百個体を完全無作為化法により圃場に栽植した。調査した 12 の農業形質は出穂期、分けつ、草丈、収量と収量構成要素および穂形質であった。各系統の再分化区と対照区で、可能な全対の形質間で相関係数をそれぞれ計算した。

再分化区では、1/4 から 1/2 におよぶ相関係数が対照区のそれらと比べて、有意に異なることが見だされた。大きさが有意に変化する相関関数の数は各品種の 2 系統で異なり、端部異質染色質の存否と関係がなかった。相関係数の変化に最も影響を及ぼした形質は出穂まで日数、草丈、植物体重および穂長であった。ほとんどの相関係数は再分化区で大きさが小さくなった。