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Herbicide response polymorphisms in wild emmer wheat: ecological and isozyme correlations

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Summary. We demonstrate that the scores and frequencies of chlortoluron (CT) and metoxuron (MX) resistance and susceptible phenotypes of wild emmer wheat, *Triticum dicoccoides*, are correlated with ecological factors and allozyme markers. Some isozyme markers located on chromosome 6B (e.g. *Adh, Est-4* and *Got*), which also harbours the CT and MX resistance gene, provide good genetic markers for herbicide resistance breeding. Significant correlations between herbicide and photosynthetic characters suggest that the evolution of herbicide resistance polymorphisms may be related to the process of photosynthesis in nature and predated domestication of cultivated wheat.

Key words: Herbicide – Polymorphism – Genetic resources – *Triticum dicoccoides* – Natural selection

Introduction

The genetic analysis and exploitation of differential responses to herbicides in crop species has recently been reviewed by Snape et al. (1991 a). The unravelling of the genetical control of such chemical response polymorphisms is crucial for breeding crops for herbicide resistance and in understanding the action and evolution of resistance in weed species. Snape et al. (1991 a) carried out tests involving difenzoquat (DF) and the three phenylureas, chlortoluron (CT), metoxuron (MX) and isoproturon (IP), and concluded from their results that the primary differences in response between resistant and susceptible varieties are due to single major genes, accompanied by modifier genes. The responses to CT and MX appear to be determined by the same gene on chromosome 6B of wheat (Snape et al. 1987; Snape and Parker 1988).

The responses of wild populations of emmer wheat, *Triticum dicoccoides*, the progenitor of all cultivated wheats (Zohary 1970), from different ecogeographical areas of Israel, to three herbicides, DF, CT, and MX, which are commonly used on cultivated wheat, were studied by Snape et al. (1991 b). Although cultivated wheats are polymorphic for response to DF, all of the genotypes of all of the tested populations of the wild species were resistant. *T. dicoccoides* was, however, polymorphic for response to both CT and MX. Our observations suggest that the genes for differential responses evolved far earlier than the domestication of the cultivated cereals and not in response to herbicide application. The results are important for breeding herbicide resistance into new varieties of cultivated wheats by classical plant breeding and/or by biotechnological means (Gasser and Fraley 1989; Schell 1987).

Linkage or the association of genetic markers to traits of agronomic importance can substantially simplify their genetic analysis, chromosomal localization, and finally, gene extraction from genetic libraries. Isozyme loci are ideal candidates for markers of quantitative genetic analysis. We have recently applied this powerful methodology to the analysis of genetic resources of wild cereals in Israel, primarily wild emmer wheat, *Triticum dicoccoides*, and wild barley, *Hordeum spontaneum*, the progenitors of cultivated wheats and barley, respectively (reviewed in Nevo 1987, 1988). Isozyme alleles and ecological factors provide an important predictive method for identifying elite genotypes characterized by single or multiple disease resistances, high protein content and a variety of quanti-

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Table 1. Geographical and climatological data for 20 populations of *Triticum dicoccoides* in Israel[a] correlated with herbicide response

Number[b]	Population	N	Ln	Rd	Huan	Dw	Sh	Th	Trd	Ev	So	Rr	Rad
1 (1)	Mt. Hermon	17	35.73	66	60	60	80	–	0	150	1	20	185[c]
5 (2)	Qazrin	24	35.67	50	58	58	50	–	60	155	5	26	189[c]
7 (3)	Yehudiyya	25	35.70	47	58	58	50	–	100	160	5	25	189[c]
9 (4)	Rosh-Pinna	20	35.52	50	58	50	75	−10	35	150	1	22	184
11 (5)	Tabigha	19	35.53	45	57	58	60	−30	120	160	5	25	188
17 (8)	Mt. Gerizim	1	35.28	47	60	42	–	10	0	155	1	25	186
18	Gitit	20	35.40	39	55	25	–	−25	100	170	1	24	195
19 (9)	Kokhav Hashahar	26	35.34	40	59	30	80	−20	25	165	1	22	195
20 (10)	Taiyiba	11	35.30	40	58	30	80	−10	25	165	1	22	190
21 (11)	Sanhedriyya	7	35.22	44	62	44	102	−10	0	155	1	21	189
22 (12)	Bet-Meir	17	35.03	44	60	61	70	−10	100	160	1	25	183
24	Amirim	10	35.45	61	60	53	85	0	13	153	1	23	182
25	Nahef	19	35.32	54	62	57	62	10	3	155	1	22	181
26	Ahihud	13	35.17	49	65	62	40	−5	20	148	1	21	180
27	Nesher	5	35.05	55	68	82	40	0	5	140	1	19	182
28	Beit-Oren	7	35.03	55	69	80	41	5	0	142	1	19	183
29	Daliyya	16	35.06	55	67	78	50	−10	100	160	2	20	181
30 (6)	Bat-Shelomo	24	35.02	55	68	77	40	−10	30	150	2	20	182
32	Yabad	12	35.15	50	63	65	52	−15	160	165	2	22	180
33	Givat-Koach	10	34.92	46	64	65	42	−20	105	160	1	26	180

Symbols of variables:
Geographical: Ln, longitude, in decimals; Temperature: Trd, mean number of tropical days; Sh, mean number of Sharav days, i.e., hot and dry days
Water availability: Rd, mean number of rainy days; Huan, mean annual humidity (%); Dw, mean number of dew nights in summer; Th, Thornthwaite's moisture index; Ev, mean annual evaporation (cm); Rr, mean relative variability of rainfall (%)
Edaphic: So, soil type: 1 = terra-rossa (= t.r.), 2 = rendzina, 5 = basalt
Solar radiation: Rad, total solar radiation per year
[a] Complete climatological data table, see Nevo and Beiles (1989)
[b] Population numbers according to Nevo and Beiles (1989); in parenthesis, the numbers according to Nevo et al. (1982)
[c] Estimated values of solar radiation by extrapolation

tative traits of agronomic importance, including germination, earliness, biomass and yield variables. In the present article we report on the application of our predictive methodology, based on ecological factors and allozyme markers, in an attempt to optimize the identification in nature of herbicide-resistant genotypes.

Materials and methods

Populations tested

The reactions for responses to the above-mentioned herbicides of 303 genotypes collected from 20 populations, across the range of *T. dicoccoides* in Israel, were tested by Snape et al. (1991 b). These populations were subdivided into three ecogeographical and climatic regions: (A) central; (B) xeric marginal, and (C) mesic marginal climatic regions. The xeric populations were further subdivided into xeric cold marginal, a northern population (Mount Hermon) (B1), and xeric warm marginal, eastern and southern populations (B2) [see Fig. 1 in Nevo and Beiles (1989) for geographical distribution].

Herbicides used and experimental techniques

The herbicides examined were the phenylurea herbicides, chlortoluron [3-(3-chloro-*p*-tolyl)-1, 1-dimethylurea], abbreviated CT, and metoxuron [3-(3-chloro-4-methoxyphenyl)-1, 1-dimethylurea], MX. To test the responses to the herbicides, individual plants of each population of *T. dicoccoides* were grown either in the field under natural conditions or in a growth room under controlled conditions, and are described in detail in Snape et al. (1991 b). In both environments, field and growth-room, qualitative (resistant or susceptible) scores were taken, relative to the performance of the controls, as well as a quantitative score of damage, on a 1–9 scale. An original score of 1 indicated no perceivable effect of the herbicide, and a score of 9, severe damage or plant death. In most experiments, it was possible to unambiguously classify individual genotypes qualitatively as being uniformly resistant, intermediate or susceptible, or segregating relative to the bread wheat controls. Frequencies of each type were based on these qualitative designations. Generally, resistant genotypes are correlated with quantitative scores of 1–3, intermediate as 4–7 and susceptible as 8–9. The CT scores are from the 1986 experiment, whereas the reaction types are from the 1987 experiment. This explains sample size discrepancies between the 2 years as well as some variation in the results. In our statistical correlation analysis of resistance with ecology, we used a reversed score as the "resistant score", where 9 represents maximum resistance.

Analysis of correlated responses

The mean responses of these *T. dicoccoides* populations to the two herbicides CT and MX, as calculated by Snape et al. (1991 b), were correlated with available ecogeographical parameters and other available genetic marker data. Geographical and climatic data for the 20 wild emmer populations tested

Table 2. Reaction of 20 populations of *T. dicoccoides* in Israel to herbicides. The populations are partitioned into three ecogeographical sets: A. central; B1. xeric-cold margin (north), B2. xeric-warm margin (east and south; C. mesic marginal (western) populations. (Numbers of populations are given in Table 1)

	Chlortoluron						Metoxuron					
	Score CTsc		Resistant CTR		Intermediate CTI frequency	Susceptible CTS frequency	Score MXsc		Resistant MXR		Intermediate MXI frequency	Susceptible MXS frequency
	n	Mean ± SD	*n*	frequency			*n*	Mean ± SD	*n*	frequency		
A. Central populations												
5. Qazrin	16	7.13 ± 1.41	0	–	–	–	24	6.83 ± 1.09	24	0.00	0.39	0.60
7. Yehudiyya	15	6.53 ± 2.26	25	0.40	0.18	0.42	25	5.28 ± 1.45	25	0.20	0.48	0.32
9. Rosh-Pinna	10	4.10 ± 2.77	15	0.67	0.07	0.27	20	5.45 ± 1.11	20	0.13	0.60	0.28
11. Tabigha	10	6.70 ± 3.09	19	0.21	0.11	0.68	19	5.05 ± 1.39	19	0.21	0.58	0.21
	51	6.27 ± 2.52	59	0.41	0.13	0.47	88	5.69 ± 1.44	88	0.13	0.51	0.36
B1. Xeric – cold marginal (northern) population												
1. Mount Hermon	13	5.15 ± 0.67	17	0.29	0.03	0.68	17	6.41 ± 1.66	17	0.06	0.44	0.50
B2. Xeric – warm marginal (eastern and southern) populations												
17. Mount Gerizim	1	5.00	0	–	–	–	1	6.00	1	0.00	0.50	0.50
18. Gitit	7	6.43 ± 2.94	0	–	–	–	20	5.35 ± 0.58	18	0.11	0.64	0.25
19. Kokhav-Hashahar	14	6.79 ± 2.78	24	0.10	0.17	0.73	26	6.27 ± 0.67	26	0.06	0.46	0.48
20. Taiyiba	9	6.67 ± 2.65	1	0.00	0.00	1.00	11	6.05 ± 0.46	11	0.09	0.50	0.41
21. Sanhedriyya	5	2.20 ± 0.45	7	0.71	0.00	0.29	7	5.36 ± 0.52	7	0.07	0.71	0.21
22. Bet-Meir	6	8.83 ± 0.45	8	0.00	0.00	1.00	17	6.26 ± 0.12	17	0.00	0.56	0.44
	42	5.11 ± 3.09	40	0.22	0.16	0.70	82	6.02 ± 0.55	80	0.05	0.53	0.41
C. Mesic marginal (wstern) populations												
24. Amirim	8	7.88 ± 1.73	9	0.00	0.22	0.78	10	6.20 ± 0.98	10	0.00	0.55	0.45
25. Nahef	12	6.67 ± 2.19	0	–	–	–	19	6.37 ± 0.83	19	0.00	0.50	0.50
26. Ahihud	8	6.63 ± 0.74	0	–	–	–	13	6.77 ± 1.17	13	0.00	0.46	0.54
27. Nesher	0	–	0	–	–	–	5	6.40 ± 0.89	5	0.00	0.40	0.60
28. Beit-Oren	0	–	0	–	–	–	7	4.86 ± 0.69	7	0.07	0.93	0.00
29. Daliyya	8	5.38 ± 1.60	10	0.40	0.25	0.35	16	5.75 ± 0.75	15	0.07	0.63	0.30
30. Bar-Shelomo	16	7.19 ± 2.01	23	0.20	0.11	0.70	24	6.65 ± 1.68	24	0.04	0.40	0.56
32. Yabad	6	3.00 ± 0.63	11	0.77	0.13	0.09	12	4.67 ± 0.78	12	0.08	0.83	0.08
33. Givat-Koach	5	5.40 ± 0.55	0	–	–	–	10	6.30 ± 1.25	10	0.00	0.45	0.55
	63	6.33 ± 2.09	53	0.32	0.16	0.52	116	6.09 ± 1.29	115	0.03	0.54	0.42
Grand mean	169	6.24 ± 2.28	169	0.32	0.12	0.56	303	5.95 ± 1.43	300	0.07	0.53	0.40
Kruskal-Wallis χ^2		0.030		5.44	2.80	8.22		3.60		12.51	0.58	1.08
between regions *P*		0.99		0.066	0.06	0.016		0.165		0.002	0.75	0.58
Chi-square χ^2		–		6.038	6.469	13.824		–		12.421	0.910	1.398
between regions df				2	2	4				2	4	4
P				0.049	0.039	0.008				0.002	0.923	0.845
Kruskal-Wallis χ^2		44.93		43.15	17.60	41.27		63.52		36.22	29.54	47.86
test between *P*		0.0002		<0.0001	0.091	<0.0001		<0.0001		0.0099	0.058	0.0003
populations *n*		169		169	169	169		303		300	300	300

appear in Table 1 and in Table 1 of Nevo and Beiles 1989. These data, derived from the Atlas of Israel (1970) and from multiple year records of the Meterological Service of Israel, were used in our correlation and multiple regression analyses in which 10–20 populations were tested, depending on the available data. Photosynthetic data are derived from Nevo et al. (1991).

Allozymic data were derived from Nevo and Beiles (1989). According to the new isozyme nomenclature our *Adh* may well be *Aadh*. Our *Ipol* is derived from leaves, and *Ipor* is derived from roots. *Got* = *Aat* in some of our previous publications.

We used SPSS-x (1986) statistical packages for conducting uni- and multivariate analyses. We preferred Kruskal Wallis non-parametric Anova, rather than the parametric Anova, and χ^2 tests for assessing the significance of the differences between regions and populations. The parametric Anova is not appropriate in our case, because the variables are not normally distributed and, more importantly, because the within-cells variances differ considerably. For example, the SD of MXsc within the regions is 1.44, 0.55 and 1.29, and the SD differences stand out even more within populations (Table 2).

Results

Ecogeographic patterns

The reaction to the two herbicides of 12–20 populations of wild emmer wheat in Israel, partitioned into the ecogeographical regions (central, marginal xeric, and marginal mesic regions), are shown in Table 2 as mean scores ± standard deviation for the two herbicides and as the frequencies of resistant, intermediate and susceptible families for CT and MX in each population. The following results are indicated.

Chlortoluron (CT) and metoxuron (MX) polymorphisms. Resistance to CT and MX was polymorphic, and significantly different between populations and between regions. The highest resistance against CT was in Sanhedriyya (near Jerusalem), which is near to Bet-Meir where the highest susceptibility was found (only 20 km apart). Similarly, the highest resistance for MX was in Yabad and the highest susceptibility in Bat-Shelomo, which are only 23 km apart. Thus, the pattern of polymorphisms is varied over very short geographic distances. This is also indicated by the frequencies of resistant, intermediate and susceptible genotypes across populations.

Geographic variation in CT and MX polymorphisms. The average level of herbicide resistance varies between regions and is significant for MXR and CTS (Table 2). All three regions harbour both resistant and susceptible populations for CT. By contrast, the range of the mean score of resistance to MX was much narrower than that for CT. From the values of MXR it is obvious that the frequency of resistant plants for MX was significantly higher in central populations as compared to ecologically marginal ones. In addition to the Kruskal Wallis tests, we conducted χ^2 tests between regions on the reaction types of CT and MX. The inter-regional differences in frequency of the reaction types to CT was significant ($\chi^2_{(6)} = 14.244$; $P = 0.027$). For MX, inter-regional differences were mainly significant for the relatively rare resistant genotypes (see MXR); the significant probability obtained ($\chi^2_{(10)} = 19.398$; $P = 0.036$) is based on small expected values, hence, should be regarded with skepticism. The inter-regional differences for CTR, CTI, CTS and MXR were all significant (Table 2). It is noteworthy that the inter-populational differences were highly significant for both CT and MX resistances measured either as scores or as frequencies (Table 2). Notably, the population of Yabad harboured significant levels of resistance for both CT and MX.

Correlation between herbicide patterns and ecogeographical parameters

Spearman rank correlations (r_s). The significant r_s correlations between MX score and climate, as well as soil types, are given in Table 3. Clearly, resistance to this herbicide increases with aridity (negative correlation between mean annual humidity, Huan and MX resistance; $r_s = -0.452^*$) and with Thornthwaite's moisture index. No significant correlation was found between CT and climate. However, CT intermediates (CTI) were negatively

Table 3. Spearman rank correlations between reaction to herbicides and ecogeographical variables, including soil type, in 20 populations of *T. dicoccoides* in Israel

Ecological variables	Symbol	*n*	Herbicide variables			
			Frequency of metoxuron resistance		Frequency of chlortoluron intermediates	
			MXR	*P*	CTI	*P*
Mean annual humidity	Huan	20	−0.452*	0.046	–	
Thornthwaite's moisture index	Th	15	−0.571*	0.017	–	
Radiation	Rad	20	0.452*	0.045		
Terra Rossa	Ter	12	–		−0.518$^+$	0.085

* $P<0.05$; $^+$ $P<0.10$

Table 4. Coefficients of multiple regression (R^2) of herbicide response variables in 10 populations of *T. diccocoides* in Israel as dependent variables and ecogeographical parameters as independent variables

Herbicide variables	Stepwise model			
	By ecogeographical variables[a]			
CTsc	Dw 0.206			
CTR	–			
CTI	Rd 0.425*	Rd Ev 0.660*	Rd Ev Rad 0.841**	Rd Ev Rad Trd 0.906**
CTS	Rr 0.206	Rr Trd 0.410	Rr Trd Sh 0.604	Rr Trd Sh Ev 0.842*
MXsc	Trd 0.328$^+$	Trd Sh 0.530$^+$	Trd Sh Rr 0.642$^+$	Trd Sh Rr Ln 0.851*
MXR	Th 0.583**	Th Ln 0.720*	Th Ln Rv 0.789*	
MXI	Trd 0.271	Trd Tj 0.627	Trd Tj Th 0.727*	Trd Tj Th Rv 0.924**
MXS	Trd 0.316$^+$	Trd Sh 0.542$^+$	Trd Sh Rr 0.723*	Trd Sh Rr Ln 0.856*

*** $P < 0.001$; ** $P<0.01$; * $P < 0.05$; $^+$ $P < 0.10$

CTsc, Score of response to chlortoluron; CTR, frequency of resistance to chlortoluron; CTI, frequency of intermediate to chlortoluron; CTS, frequency of susceptible to chlortoluron; MXsc, score of response to metoxuron; MXR, frequency of resistance to metoxuron; MXI, frequency of intermediate to metoxuron; MXS, frequency of susceptible to metoxuron

[a] Symbols of ecogeographical variables; see Table 1

Table 5. Spearman rank correlations between variables of response to herbicides in 9–20 populations of *T. diccocoides* in Israel and allozyme frequencies[a]

Allele		*n*	CTsc	CTR	CTI	CTS	MXsc	MXR	MXI	MXS
n:			18	12	12	12	20	20	20	20
Got-1B	a	20			−0.644*					
Got-3A	a	20								0.378 +
Acph-3	b	20					−0.495*	0.528*		0.466*
Adh-1B	a	20	−0.427 +	−0.667*		0.631*				
Est-4A	a	20								0.378 +
Est-4B	Null	20			0.666*				0.432*	
Est-5A	a	20						0.464*		−0.383 +
	c	20			0.655*					
	d	20	−0.615**				−0.413 +	−0.380 +	0.449*	0.448*
Gdh-B	b	20						0.393 +	0.467*	−0.444*
Gluc-B	a	15			0.892***					
Hk	b	16					−0.577*	−0.660**		0.431 +
	c	16					0.492*	0.583*		
Ipol	b	18					0.611*	0.513*	0.647**	−0.728***
	c	18					−0.522*	−0.580*	−0.512*	0.657**
Mdh-1A	a	20						0.394 +		
Pgi-A	e	20		−0.620*		0.598*				
6Pgd-1B	b	20							0.407*	
6Pgd-2	a	20					−0.411 +	−0.701**		
	b	20						0.391 +		
	c	20					0.431 +	0.640**		

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; + $P < 0.10$

CTsc, Score of response to chlortoluron; CTR, frequency of resistance to chlortoluron; CTI, frequency of intermediate to chlortoluron; CTS, frequency of susceptible to chlortoluron; MXsc, score of response to metoxuron; MXR, frequency of resistance to metoxuron; MXI, frequency of intermediate to metoxuron; MXS, frequency of susceptible to metoxuron

[a] Allzymic data derived from Nevo and Beiles (1989). According to the new isozyme nomenclature our *Adh* may be *Aadh* and *Got* = *Aat*

correlated with terra-rossa soil type ($r_s = -0.518^+$), i.e. they were primarily found in plants growing on either rendzina or basalt. The frequency of MXR was also correlated with the second factor of temperature variables, derived by Principle Component analysis, representing 21.7% of the variance ($r_s = 0.448$ *).

Multiple regression analysis. We conducted a stepwise multiple regression analysis (SPSSx 1986), employing herbicide parameters as dependent variables and ecogeographical variables as independent ones, in an attempt to explain the variances in herbicide patterns (Table 4). A substantial amount of herbicide variances in susceptibility or resistance is largely ($R^2 = 0.79 - 0.94$) and significantly ($P > 0.05 - 0.01$) explained by 1–4 ecogeographical variables. For example, the variance in MX resistance is largely and significantly explained ($R^2 = 0.79$ *) by two climatic variables and longitude: Thornthwaite's moisture index alone explains a high amount of the variance ($R^2 = 0.58$ **). Clearly, herbicide polymorphisms are intimately associated with climatic and soil variability. The main climatic variables explaining herbicide variances were: number of tropical days, Sharav (number of hot and dry days), rainfall and evaporation; i.e. variables representing moisture and/or aridity stress.

Correlation between herbicide patterns and allozyme markers, and their chromosomal localization

Spearman rank correlations (r_s). The r_s values and their significance of herbicide scores and frequencies with allele frequencies at 11 loci are given in Table 5. Clearly, the substantive number of significant correlations suggest that some allozyme loci are good potential genetic markers for herbicide resistance. This is particularly true for the MX score and frequencies as well as for CT. The major loci providing good markers are hexokinase (*Hk*), alcohol dehydrogenase (*Adh*), esterases-4,5 (*Est*-4,5), acid phosphatase (*Acph*-3), glutamate-oxaloacetate transaminase (*Got*) and indophenol oxidase of leaves (*Ipol*). Most remarkably, *Adh*, *Got* and *Est*-4 are located on chromosome 6, the chromosome to which CT and MX resistances have been assigned (Snape et al. 1987). *Hk* is located on chromosome 3, and *Acph*-3 on chromosome 4 (Hart and Gale 1989).

Multiple regression analysis. A stepwise multiple regression (MR) analysis (SPSSx 1986) on the herbicide variables as dependent variables and multilocus allozyme frequencies as independent variables was carried out (Table 6). A substantial amount of the variance in herbi-

cide variation is largely ($R^2 = 0.39-0.86$) and significantly ($P < 0.05 - 0.001$) explained by 1–3 allozyme multilocus combinations (Table 6). For example, a two-variable allozyme combination (*6pgd*-2[a] and *Ipol*[c]) explains ($R^2 = 0.86$***) the variance in the frequency of resistant MX plants. Likewise, a two-variable combination explains ($R^2 = 0.82$**) the variance in CTI. The main isozyme variants explaining herbicide patterns were those that showed earlier significant correlations in the Spearman rank correlations, i.e. *Ipol*, *Acph*-3, *Est-5A*, 5B[a], *6Pgd*-2, *Gluc*-B[a] and *Adh*-1B[a].

Table 6. Coefficients of multiple regression (R^2) of herbicide response variables as dependent variables and allozyme frequencies[a] in 9–20 populations of *T. diccocoides* in Israel as the independent variables. For herbicide abbreviations see Table 4

Herbicide variables	Stepwise model			
	By allozyme frequencies n			
CTsc	18	Adh-2B a 0.185 +	+Est-5A d 0.281 +	+Est-5B a 0.394 +
CTR	9	–		
CTI	9	Gluc-B a 0.494*	+Adh-1B a 0.820**	
CTS	9	–		
MXsc	16	Est-5A d 0.288*	+Acph-3 a 0.451*	
MXR	11	6Pgd-2 a 0.802***	+Ipol c 0.864***	
MXI	11	Ipor-B a 0.297 +	+Est-5A d 0.498 +	+Pgm-A a 0.637*
MXS	11	Ipol b 0.396*	+6Pgd-2 a 0.561*	+Ipor-B a 0.757**

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; + $P < 0.10$

[a] Allozymic data derived from Nevo and Beiles (1989). According to the new isozyme nomenclature our *Adh* may be *Aadh* and *Got* = *Aat*

Correlation between herbicide variation and photosynthetic performance

Several interesting Spearman rank correlations were found between mean population herbicide score and frequencies in 12–20 populations of *T. dicoccoides* and mean parameters of photosynthetic performance derived earlier (Carver and Nevo 1990; Nevo et al. 1991) (Table 7). CT-sucseptibility (CTS) frequency was correlated with CO_2 assimilation rate per chlorophyll concentration (A/Chl) ($r_s = -0.574$*), and CTI frequency with internal CO_2 concentration (Ci), stomatal conductance to water vapour (gs) and total chlorophyll concentration (Chl) ($r_s = -0.641$*; -0.676* and -0.761**, respectively).

Discussion

Herbicide polymorphisms in wild emmer: ecogeographical patterns

Our data clearly demonstrates that wild emmer shows polymorphic responses to the two herbicides, CT and MX, commonly used on cultivated wheat. This suggests that the genes for differential response have evolved prior to the domestication of cultivated wheats, and not in

Table 7. Spearman rank correlations between variables of response to herbicides in 12–20 populations of *T. diccocoides* in Israel and parameters of photosynthetic performance. All coefficients larger than 0.25 appear in the table

Photosynthetic parameters	CTsc	CTR	CTI	CTS	MXsc	MXR	MXI	MXS
n:	18	12	12	12	20	20	20	20
A		−0.272	−0.535 +	0.312				
A/Chl		0.533*	0.556 +	−0.574*				
LA		0.526 +	0.373	−0.553 +		0.344		−0.265
Ci			−0.641*				0.289	
WUE		0.363	0.556 +	−0.424				
g_s			−0.676*				0.265	
Chl-a		−0.437	−0.676*	0.490				
Chl-b			−0.338			0.431 +		
Chl-total		−0.349	−0.761*	0.414				

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; + $P < 0.10$

Herbicide variables: CTsc, Score of response to chlortoluron; CTR, frequency of resistance to chlortoluron; CTI, frequency of intermediate to chlortoluron; CTS, frequency of susceptible to chlortoluron; MXsc, score of response to metoxuron; MXR, frequency of resistance to metoxuron; MXI, frequency of intermediate to metoxuron; MXS, frequency of susceptible to metoxuron

Photosynthetic parameters: A, CO_2 assimilation rate (leaf area basis); A/Chl, CO_2 assimilation rate (chlorophyll basis) ; LA, single leaf area; Ci, internal CO_2 concentration; WUE, water use efficiency; g_s, stomatal conductance; Chl-a, chlorophyll a concentration; Chl-b, chlorophyll b concentration; Chl-total, total chlorophyll concentration

response to the development and use of the chemicals (Snape et al. 1991 a, b). It is remarkable that the distinct resistance to CT is concentrated mainly in two populations: Sanhedriyya and Yabad. Even more striking is the fact that a highly resistant population for CT (Sanhedriyya) is separated by only 20 km from a highly susceptible population (Bet Meir), while much smaller differences exist among remote populations. This suggests the operation of natural selection across short geographical distances and/or stochastic changes in gene frequencies in local populations. However, in a stochastic process we do not expect the largest difference to occur between neighbours. Hence, natural selection appears to be the more likely explanatory model. Similar contrasts over short geographic distances occur between CT-resistant Yabad and CT-susceptible Bat-Shelomo. A less dramatic differentiation occurs between MX intermediate Beit-Oren, a small, highly mesic marginal population on Mt. Carmel, and the MX-susceptible Bat-Shelomo population; these populations are separated by about 15 km. We have earlier shown that response to CT and MX is under a common genetical control in most populations, (Snape et al. 1991 b), although there may also be genes for independent response in certain populations. This may explain the differential response of CT and MX in this study. The Spearman rank correlation between the mean score of MX and CT was $r_s = 0.456$; $P = 0.056$ ($n = 18$); between the frequencies of resistance to MX and CT, $r_s = 0.512$; $P = 0.089$ ($n = 12$). This indicates a moderate level of association between the resistances of MX and CT. Hence, either there are more loci involved in MX or CT resistance, or there might be a differential influence of different alleles on the resistance. This result makes the Rosh Pinna and Yehudiyya populations promising potential genetic resources for herbicide resistance because they harbour high frequencies of resistance for the two herbicides (high CTR and MXR, Table 2). All in all, herbicide polymorphisms appear to be distinctly patterned ecogeographically and exploitable in nature.

Ecology and allozyme predictability of herbicide responses in wild emmer

The herbicides are clearly patterned ecogeographically in wild emmer, either with climate, soil, or additional yet unidentified factors, possibly those including climatic unpredictability (e. g. in the Gitit population). Most importantly, the high frequency of MX resistance and CT intermediates are highly and significantly correlated with a combination of climatic factors, primarily those related to the moisture climatic regime (i. e. Thorntwaite's moisture index, Th; number of rainy days, Rd; relative humidity, Huan; evaporation, Ev) and/or temperature (i. e. number of tropical and Sharav days, Trd and Sh, respectively). The lack of massive climatic correlates with CT apparently derives from the patchiness of resistant types over very short geographical distances (see Results and Tables 2 and 3). Therefore, microecological factors may override the macroecological ones used in our correlation study. We found earlier (reviewed in Nevo 1987) that allozyme markers and ecological factors provide an important predictive method for identifying elite genotypes for diverse characters of agronomic importance. Herbicide resistance polymorphisms, like all of the other characters we studied (disease resistances, agronomic traits, protein level, photosynthetic yield etc.) can be predicted in nature, hence optimizing sampling strategies. Herbicide polymorphisms abound in drier environments and/or rendzina and basalt soil types, and can be identified by single and multilocus structures.

Most importantly, we found that several isozyme loci (e. g. *Adh*, *Got* and *Est*-4) located on chromosome 6 (Hart and Gale 1989), which also harbours the resistant gene to CT and MX (Snape et al. 1987), are highly and significantly correlated with herbicide resistance (scores and frequencies) (Tables 5 and 6). Hence, isozymes and DNA genetic markers, such as restriction fragment length polymorphisms (RFLPs) could be used for precise chromosomal localization of the herbicide genes. This will facilitate its extraction from genetic libraries and will allow (1) the unraveling of the molecular, biochemical and physiological structure and function of the gene, and (2) its Utilization for transgenic manipulation in breeding. In our study several other isozyme loci were also highly correlated with herbicide resistances (*6Pgd*, *Pgi*, *Hk*, *Gdh*, Est-5, *Acph* and *Ipol*; Tables 5 and 6). These may either be due to linkage disequilibrium with the genes located on chromosome 6 and/or with other chromosomes that may carry modifiers or even additional major urea-herbicide resistant genes.

Herbicide response correlation with photosynthetic performance

The herbicide resistance polymorphisms in wild emmer indicate that their evolution predated the domestication of wheat. Urea herbicides are known to inhibit photosynthesis by inhibiting electron transfer at a site near photosystem II. Thus, urea herbicides are intimately linked with the photosynthetic process. The correlation that we found between herbicide patterns and photosynthetic performance in wild emmer may indeed suggest that urea-herbicide evolution was directly or indirectly related to adaptive evolution of photosynthesis. Thus, herbicide genes are not only providing good genetic markers for wheat mapping, but they may also be viewed as adaptive genes for biochemical pathways associated with photosynthesis, DNA synthesis and other organic processes.

Conclusions and prospects

Wild emmer wheat, *Triticum dicoccoides*, the progenitor of all cultivated wheats, harbours rich genetic resources for wheat improvement in its center of origin and diversity in Israel (reviewed in Nevo 1988). Wild emmer contains genetical, morphological, biochemical, physiological, immunological and agronomic traits appropriate for wheat improvement. Recently, we added herbicide resistance to the list of potential, largely untapped, genetic resources of wild emmer (Snape et al. 1991 a, b; the present study). Herbicide response polymorphisms in wild emmer are ecogeographically patterned and predictable by ecological and allozyme markers, some of the latter being located on chromosome 6 together with the resistant gene of CT and MX. Furthermore, herbicide resistance in wild emmer is correlated with variation in photosynthetic performance (described by Carver and Nevo 1990; Nevo et al. 1991). The aforementioned facts suggest that herbicide resistance in wild emmer predated wheat domestication and that it presumably co-evolved adaptively with photosynthetic capacity.

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