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Action of elevated CO_2 and high temperatures on the mineral chemical composition of two varieties of wheat

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INTRODUCTION. – It seems that the future climate change will be accompanied by increased carbon dioxide levels in the atmosphere, which will probably double before the end of the next century (MURRAY, 1995). Temperature is also expected to increase. It is therefore necessary to know the effects that such changes will have on plants so that their cultivation can be adapted to the new circumstances with a view to taking maximum advantage of the new climatic conditions.

The most recent works published in this direction have generally addressed the repercussions that these factors will have on photosynthesis and related aspects in different types of plants.

Cereals, and wheat in particular, have been addressed in such studies. High CO_2 levels were found at anthesis to increase the weight of the stem and ear, and in maturity it increased biomass and yield, the latter because of the number of grains per spikelet and per ear, and the number of stems with ears increased (MULHOLLAND *et al.*, 1994). THOMPSON and WOODWARD (1994) also observed a 30% increase in the yield of wheat grain. Long-term, the increase in CO_2 decreased photosynthetic capacity (DELGADO *et al.*, 1994), although photosynthesis was greater than that measured with the lowest concentration of CO_2 (ANDRE and DUCLOUX, 1993).

There are fewer references in the literature, however, to the action of these factors on nutrients, inside or outside wheat plants.

MIGLIETTA *et al.* (1996) did not observe any significant effect on the photosynthetic capacity of leaves well fertilized with N, indicating that this nutrient plays an important role in the effects of CO_2. Owing to the increase in soluble carbohydrates, fructans and stem growth brought about by increased CO_2, the critical and minimum concentrations of leaf N decrease, as does the N stored in the leaves, the N in the stem and grain – with the corresponding losses of quality – and also Ca, S, Mg and Zn (FANGMEIER *et al.*, 1997; ROGERS *et al.*, 1996). High atmospheric CO_2 also produces plants of inferior quality, with lower N contents, especially in C3 plants, also affecting the distribution of this element, N contents being more reduced in the above-ground parts than in the roots (Cotrufo *et al.*, 1998).

In the light of all these findings, in the present work we were prompted to explore the response that the different parts of two different varieties of wheat plants show in pre-anthesis and at harvesting to a higher CO_2 level and a higher temperature than normal as regards their mineral contents.

Materials and methods. – To cultivate plants in an atmosphere with a higher CO_2 content and a higher temperature than normal we used temperature gradient tunnels (Rawson *et al.*, 1995) on the crop, shortly after emergence. This allowed the cultivation of spring wheat at two different CO_2 concentrations – ambient, 350 ppm in one tunnel – and 700 ppm in the other one. Two varieties were cultured in each tunnel – Alcázar, which is more precocious, and Rinconada – at two temperatures: ambient and 4°C higher with three replicates. Each variety occupied a longitudinal half of each tunnel, which measured 9 m in length, 2 m in width and 1.3 m in height. The three replicates designed were also distributed longitudinally. Each tunnel comprised three transverse modules, which were separated by polycarbonate septa with horizontal slits to decrease the mixing of air between the modules. The rest of installations were ventilators, probe Pt-100 systems, integrators and differentials (PID). The data were recorded continuously on a computer. Thus, treatments were maintained during the assays.

The soil is in a cereal-growing area. It is acid, fine-sandy at the surface and clayey at depth, poor in organic matter, highly evolved, and poor in mineral elements. Fertilisation, which was equal throughout the experiments, consisted of the application of N, P and K (32, 60 and 60 kg ha^{-1}, respectively) on 21 October 1996, plus 400 kg/ha^{-1} of 26% calcium ammonium nitrate on 6 February 1997, shortly before sowing, which took place 11 days later at a ratio of 180 kg ha^{-1} of seeds. Irrigation corresponded to what plants cultivated in the zone would receive, calculating the monthly means with the data of 20 years and applying the water weekly by drip irrigation.

Two samples of plant material were collected. Pre-anthesis, 27 May, differentiates both varieties somewhat owing to the different precocities of both varieties. The Alcázar variety was more precocious. The stems were counted in 0.5 m of two adjacent rows in three different sites of the culture of each combination of variety, temperature and CO_2 level and five consecutive stems were harvested from each end of the 0.5 m portion of the two rows. Then, the fresh weight of the samples was obtained and the following parts were separated: the *flag leaf, rest of leaves*, the *ear*, the *last internode* and the *rest of stem*, with the sheaths of the leaves, whose green surfaces were measured with a photoelectric planimeter. At harvesting, at the end of the cycle, 0.5 m of two adjacent rows was harvested in each of the three replicates and the fresh weight was obtained. In a subsample of known weight the stems were counted, separating the following parts: *grain*, glumes and rachis (*glumes+rachis*) and the stem with leaves (*straw*). After a 48 h drying period at 50°C, the dry weight of the different parts of the plant in the two samples was obtained; the names in italics are readily associated with the corresponding phase of the culture.

The N content was determined using a specific ammonium electrode after Kjeldhal digestion. Another ground plant material subsample was mineralised in an oven at 450°C and then dissolved in 25% HCl and brought up to volume. Phosphorus analysis was based on colorimetric measurement (400 nm) of vanadomolybdophosphate. The other nutrients were determined using an atomic absorption spectrophotometer.

For each of the parts of the plant, statistical analysis involved the following: mean, standard deviation and coefficient of variation, maximum and minimum values, together with analysis of variance, as in a three-block factorial experiment, whose sources of variation were varieties, CO_2 concentrations, temperatures and replications and interactions.

RESULTS AND DISCUSSION. – The mean values of the three replicates of the results, including, as well as the nutrients, the dry weight are shown in Tables 1 and 2. The differences obtained in the analyses of variance and of their significance are shown in Table 3, in which, overall, a high degree of significance can be appreciated. Unless otherwise stated, only results with a $P < 0.01$ are discussed.

Dry weight. – The mean values of this parameter range between 46 and 390 g m^{-2} in the different parts of the plant a few days before flowering (27 May). At harvest, the range is 226 and 1025 g m^{-2}.

The high CO_2 concentration decreased dry weight per square meter of the leaves, the fractions with the lowest weight: *flag leaf* and *rest of leaves*. The decrease in transpiration and in the surface of the leaves due to the high CO_2 concentration, observed by ANDRE and DUCLOUX (1993), could be associated with this result.

In comparison with the normal level, in the Rinconada variety, with a slightly longer cycle, high CO_2 only decreased dry weight in the parts of the plant corresponding to harvesting *grain, straw* and *glumes+rachis*. MULHOLLAND *et al.* (1997) in the Minaret variety, reported increases in the total and grain biomass. Starting out with an ambient CO_2 level, high temperature also decreased *grain* weight in both varieties. Working with winter wheat, DELGADO *et al.* (1994) reported that high temperature reduces the protein content of the plants, including Rubisco, chlorophyll, and photosynthetic capacity, shortening the effective life of the leaves.

Nutrients. – The reaction of the nutrients to these factors was very different and was in general characterised by being independent of the above-mentioned fluctuations in dry matter.

The mean values of N concentrations in the samples taken during the pre-anthesis period ranged between 0.76 and 4.67% and between 0.43 and 2.60% at harvest.

The Alcázar variety was found to have more N than the Rinconada

TABLE 1. – *Pre-anthesis.*

Organ	Variety	CO_2	Temperature	N %	P %	K %	Ca %	Mg %	Fe ppm	Mn ppm	D w gm-2
Flag leaf	Alcazar	Ambient	Actual	4.62	0.41	2.66	0.87	0.29	117	54	55
			+4°C	4.60	0.35	2.76	0.77	0.29	104	55	65
		Elevated	Actual	4.28	0.35	2.41	0.87	0.31	117	65	46
			+4°C	3.52	0.31	2.45	0.73	0.31	88	16	72
	Rinconada	Ambient	Actual	4.20	0.33	2.37	0.70	0.26	112	39	76
			+4°C	4.10	0.32	2.56	0.60	0.28	99	33	70
		Elevated	Actual	4.67	0.37	2.51	0.55	0.24	105	20	59
			+4°C	4.49	0.36	2.64	0.61	0.22	104	25	50
Rest of leaves	Alcazar	Ambient	Actual	3.86	0.42	3.28	0.89	0.33	110	29	86
			+4°C	4.36	0.44	3.31	0.81	0.33	97	29	100
		Elevated	Actual	3.63	0.47	2.97	0.83	0.31	101	27	77
			+4°C	4.07	0.32	2.90	0.87	0.34	85	34	109
	Rinconada	Ambient	Actual	4.26	0.38	3.07	1.00	0.48	166	32	114
			+4°C	4.23	0.40	3.07	0.70	0.40	91	22	81
		Elevated	Actual	3.74	0.45	3.09	0.81	0.40	139	17	78
			+4°C	3.78	0.35	2.85	0.64	0.31	93	25	56
Ear	Alcazar	Ambient	Actual	2.09	0.30	2.03	0.12	0.09	98	6	187
			+4°C	1.99	0.28	1.79	0.13	0.08	113	5	212
		Elevated	Actual	2.32	0.33	1.82	0.11	0.09	83	6	175
			+4°C	2.37	0.26	1.76	0.12	0.09	99	6	185
	Rinconada	Ambient	Actual	1.96	0.32	2.09	0.04	0.08	46	3	223
			+4°C	2.09	0.35	2.15	0.04	0.07	63	4	175
		Elevated	Actual	1.99	0.35	2.11	0.08	0.07	59	5	200
			+4°C	2.31	0.35	2.08	0.06	0.08	96	13	175
Last internode	Alcazar	Ambient	Actual	1.79	0.31	2.82	0.22	0.14	101	42	134
			+4°C	1.77	0.33	2.85	0.24	0.15	106	20	171
		Elevated	Actual	1.70	0.31	2.79	0.21	0.14	110	19	120
			+4°C	1.62	0.28	2.76	0.19	0.12	82	17	187
	Rinconada	Ambient	Actual	1.77	0.26	2.67	0.18	0.10	78	14	152
			+4°C	2.13	0.33	2.92	0.24	0.14	88	23	122
		Elevated	Actual	2.01	0.35	2.91	0.20	0.12	81	22	137
			+4°C	2.11	0.34	2.89	0.18	0.09	71	19	122
Rest of stem	Alcazar	Ambient	Actual	0.83	0.19	2.70	0.09	0.14	121	15	274
			+4°C	1.04	0.22	3.07	0.11	0.12	116	18	308
		Elevated	Actual	0.78	0.17	2.42	0.08	0.11	120	10	284
			+4°C	0.77	0.19	2.46	0.08	0.09	128	8	390
	Rinconada	Ambient	Actual	0.90	0.20	2.80	0.17	0.09	148	15	342
			+4°C	1.03	0.24	3.45	0.13	0.09	169	19	222
		Elevated	Actual	0.76	0.23	2.96	0.11	0.06	139	20	266
			+4°C	0.92	0.25	2.92	0.09	0.04	108	17	217

TABLE 2. – *Final harvest.*

Organ	Variety	CO_2	Tempe-rature	N %	P %	K %	Ca %	Mg %	Fe ppm	Mn ppm	D w gm 2
Grain	Alcazar	Ambient	Actual	1.89	0.30	0.56	0.14	0.11	20	9	1025
			+4°C	2.35	0.36	0.77	0.13	0.14	19	9	545
		Elevated	Actual	2.16	0.34	0.82	0.14	0.11	20	8	742
			+4°C	1.87	0.35	0.73	0.17	0.13	25	10	894
	Rinconada	Ambient	Actual	2.21	0.32	0.91	0.22	0.11	22	14	963
			+4°C	2.60	0.36	0.82	0.17	0.13	22	17	803
		Elevated	Actual	2.00	0.29	0.62	0.14	0.09	23	13	634
			+4°C	2.06	0.37	0.79	0.14	0.09	16	13	597
Straw	Alcazar	Ambient	Actual	0.55	0.10	2.77	0.49	0.17	203	25	785
			+4°C	0.68	0.09	2.69	0.40	0.12	163	15	645
		Elevated	Actual	0.53	0.10	2.40	0.38	0.10	221	18	971
			+4°C	0.50	0.09	2.48	0.39	0.11	187	12	709
	Rinconada	Ambient	Actual	0.59	0.07	2.63	0.43	0.12	156	13	784
			+4°C	0.71	0.09	2.98	0.40	0.11	166	14	787
		Elevated	Actual	0.43	0.10	2.47	0.38	0.08	132	9	464
			+4°C	0.56	0.13	2.71	0.37	0.08	195	13	623
Glumes+ rachis	Alcazar	Ambient	Actual	0.67	0.08	1.69	0.20	0.03	149	5	368
			+4°C	1.12	0.16	1.57	0.23	0.06	61	14	284
		Elevated	Actual	0.81	0.17	1.20	0.18	0.03	49	14	380
			+4°C	0.56	0.11	1.29	0.21	0.04	84	14	345
	Rinconada	Ambient	Actual	0.60	0.09	1.32	0.19	0.03	85	13	354
			+4°C	1.00	0.15	1.00	0.20	0.07	120	35	413
		Elevated	Actual	0.54	0.11	0.94	0.19	0.03	88	13	226
			+4°C	0.56	0.17	1.09	0.17	0.03	113	19	288

variety in *glumes+rachis*. High CO_2 increased N in the *ear* but decreased the amounts of this element in the *rest of leaves, rest of stem* and *straw* fractions. ROGERS *et al.* (1996) also observed this decrease in the leaves and stem of wheat. High temperature, by contrast, elicited a greater concentration of this nutrient but only in the *straw* fraction.

In the Alcázar variety, elevated CO_2 led to a decrease in N in the *flag leaf* and in *the last internode*, consistent with the results of the researchers cited immediately above. In Rinconada, high temperature increased this nutrient in the *last internode*. Finally, when CO_2 was present at ambient concentration, high temperature increased the concentration of N in the *glumes+rachis*.

TABLE 3. – *Analysis of variance - Probabilities.*

	Source Vari	Pre-anthesis					Final harvest		
		Flag leaf	Rest leaves	Ear	Last intern	Rest stem	Grain	Straw	Glum+ rach
Dry weight	Variety (A)					0,0239			
	CO_2 (B)	0.0078	0.0029						
	A*B						0.0009	0.0007	0.0002
	A*C		0.0443					0.0171	0.0252
	B*C						0.0071		
Nitrogen	Variety (A)								0.0003
	CO_2 (B)		0.0002	0.0023		0.0015		0.0005	
	Temperat (C)					0.0196		0.0019	
	A*B	0.0001			0.0003				
	A*C		0.0452		0.0030				
	B*C								0.0001
	A*B*C						0.0271		
Phosphorus	Variety (A)			0.0001		0.0090			
	A*B	0.0181						0.0210	
	A*C							0.0228	
	B*C		0.0013						
	A*B*C								0.0019
Potassium	Variety (A)			0.0001		0.0001			0.0001
	CO_2 (B)		0.0012					0.0001	0.0005
	A*B	0.0171					0.0399		
	A*C							0.0093	
	B*C					0.0270			
Calcium	Variety (A)	0.0004							0.0076
	CO_2 (B)				0.0165	0.0001		0.0002	0.0200
	Temperat (C)		0.0424						
	A*B			0.0078			0.0001		
	A*C					0.0478	0.0387		
	B*C						0.0366		
Magne-sium	Variety (A)	0.0035	0.0002	0.0008		0.0001		0.0047	
	CO_2 (B)		0.0134		0.0214	0.0001		0.0002	
	Temperat (C)					0.0353			
	A*B						0.0299		
	A*C						0.0026		
	B*C						0.0059		
	A*B*C								0.0129
Iron	Variety (A)				0.0004				
	Temperat (C)		0.0397	0.0264					
	A*B			0.0397					
Manga-nese	Variety (A)	0.0001					0.0001		
	CO_2 (B)							0.0021	
	A*B			0.0168		0.0094			0.0002
	A*C							0.0252	0.0042
	B*C			0.0420					0.0037

Nitrogen showed a more frequent response with respect to the other nutrients, corresponding to the most pronounced participation of the element in photosynthesis and in the formation of carbohydrates and proteins related to the process. The increase in N levels in the *ear* fraction due to the effect of the high CO_2 level could be due to a different distribution in the plant since according in the analysis of variance also carried out for this parameter total N was not altered. Under the assay conditions employed, it is possible to observe how the two varieties employed vary; that is how the elevated CO_2 concentration led to a general decrease in the content of N and increased it with a slightly higher ambient temperature. COTRUFO et al. (1998) proposed that elevated CO_2 levels would decrease plant quality owing to a decrease in N levels in the tissues, although different effects on the N concentration in different types of plant appear.

The concentration of Mg in samples from the pre-anthesis period ranged between 0.04 and 0.48% while in those collected at harvesting it ranged between 0.03 and 0.17%.

The concentration of Mg afforded highly significant differences in both varieties, this possibly being the best characteristic to differentiate between them. Thus, the Alcázar variety was seen to have a higher content in Mg in *flag leaf, ear, rest of stem* and *straw*, whereas in Rinconada the same effect was observed in *rest of leaves,* which could be related to the different precocity seen in the two varieties.

With a high CO_2 level with respect to the normal one, the content of Mg in both varieties was lower in the *rest of stem* and *straw* fractions. FANGMEIER *et al.* (1997) reported such a decrease in this nutrient but referring to the *grain*, as in the case of N, with a loss of quality. In the Alcázar variety, the content in this nutrient in *grain* increased due to the action of high temperature and in both varieties when starting out with a normal CO_2 atmospheric level.

As in the case of N, the Mg content seems to be more regulated than other nutrients by the variables being studied, indicating the great importance of Mg in the evaluation of possible climate changes, this is even more prominent if it is taken into account that the element is a macronutrient that is not applied in fertilisation.

Potassium, Ca and Mn form a cationic group that would follow in importance owing to the frequency of significant differences in their contents caused by the factors under study. The mean K values ranged between 1.76 and 3.45% in pre-anthesis and between 0.56 and 2.98% at harvesting. Those of Ca ranged between 0.04 and 1.00% in the pre-flowering period and between 0.13 and 0.49% at harvesting. The values for Mn were between 3.5 and 65 ppm and 5.1 and 35 ppm, respectively.

The variables that, in a relative fashion, most affect the statistical differences in the contents of these elements in the plant are variety and the CO_2 level.

The varieties differ between each other in the K concentration, which in the Rinconada variety was greater in the *ear* and *rest of stem,* while in the Alcázar variety it was greater in *glumes+rachis.* In the Alcázar variety, Ca was higher in the *flag leaf* and in *glumes-rachis,* while the Mn content in this variety was higher in the *flag leaf* fraction and, in Rinconada, in *grain.* These results confirm the importance of variety when evaluating the chemical composition of the different fractions and its alteration due to the variables studied.

Elevated CO_2 elicited a lower content in these cations: of all three in *straw* and also of K in the *rest of stem* and *glumes+rachis* fractions and of Ca in *rest of stem.* This decrease in minerals is only comparable with the above-described decrease in N and might also have repercussions in plant quality (COTRUFO *et al.*, 1998).

In the Rinconada variety, high CO_2 increased the Ca level in *ear,* in pre-anthesis, and decreased it in *grain* at harvest. Mn decreased in *rest of stem* in Alcázar, whereas in Rinconada it decreased in *glumes+rachis.* In Rinconada high temperature increased K in *straw*, and Mn in *glumes+rachis.* Finally, with a normal atmospheric CO_2 level high temperature increased Mn levels in *glumes+rachis* in both varieties.

Phosphorus showed very few changes due to the variables explored, with a range between 0.07 and 0.47% for all the samples analysed.

The *ear* and *rest of stem* fractions had more P in the Rinconada variety. When CO_2 was elevated, high temperature decreased P in the *rest of leaves* fraction. Only in Alcázar and at the low ambient CO_2 concentration did high temperature increase P in *glumes+rachis.* This poor response coincides with the observations of FANGMEIER *et al.* (1997) who indicated that concentrations in green tissues were not affected, or only slight affected, by the action of elevated CO_2.

Iron, the element that least changes in the plant due to the action of the variables studied, occupied the last position. The range of values lay between 16 and 221 ppm for all the samples.

Variety was found to be the most important variable. In the *stem* fraction the Fe level of the Alcázar variety was higher than that seen in Rinconada, a further difference for differentiating between the two varieties.

In sum, the nutrients N and Mg are the most sensitive to being differentiated in the plant owing to the action of the variables studied: variety, CO_2, temperature and interactions. Iron is less sensitive and so, although to a lesser extent, is P. Fluctuations in dry matter have very little effect on those of the nutrients, which are characterised by showing their own type of response.

The variety of the plant is the factor with the greatest effects, closely followed by the CO_2 concentration, while temperature has little effect. With respect to the different plant fractions, those of *glumes+rachis* and *straw* are the most sensitive, followed by *rest of stem* and *grain.*

Variety differences are essential, completely different reactions being obtained in each of them, the nutrients and affected plant parts being divided between them, as reported. In general, the Alcázar variety can be said to have more N, Mg and Ca in some of its fractions whereas in Rinconada this is the case of P and K. The action of high CO_2 is manifest in a general decrease in the content of the nutrients, in consonance with the reports of other authors (CAO and TIBBITS, 1997; COTRUFO *et al.*, 1998; FANGMEIER *et al.*, 1997; ROGERS *et al.*, 1996; THOMPSON and WOODWARD, 1994), although more commonly in the case of N, K, Ca and Mg. The higher temperature had an effect, mainly in an increase in N in the *straw* fraction.

Under the conditions of the experiments, N and Mg were seen to be the elements that showed the greatest response to the action of climate change. The reactions of the photosynthetic process and carbohydrate levels to the variables studied, reported in the Introduction, are strongly related to these two key elements in these processes.

CONCLUSIONS. – From the above it may be deduced that the variety of the plant is a very important factor to be considered in the cultivation of wheat under future climatic conditions. The *grumes+rachis* and the *straw* fractions may be the parts of the plant whose chemical composition best expresses the action of the variables, followed by *rest of stem.* The most sensitive plant nutrients are N and Mg, elements that have already been reported to play very important roles in the photosynthesis process and related phenomena (TAYLOR *et al.*, 1994; MULHOLLAND *et al.*, 1997).

For the cultivation of wheat under future climatic conditions it may be important to take into account the variety of the plant and N and Mg fertilization.

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SUMMARY. – Two varieties of spring wheat were cultured in temperature gradient tunnels with two CO_2 concentrations and two different temperatures. We discuss the effects of these three variables on the mineral chemical composition of the plant samples, separated into fractions, taken during two phases: a few days before anthesis and at harvesting.

The variety of the plant and the CO_2 concentration were the variables found to exert the greatest influence, the glumes and rachis, the straw and the rest of the stem (without the last internode) being the parts of the plant that most frequently showed different values owing to the effect of the variables. The nutrients N and Mg were the most sensitive to differentiation in the plants owing to the action of the variables. Iron was seen to be less sensitive, as was P.