Relationship between Grain Yield, Osmotic Adjustment and Benzoxazinone Content in *Triticum aestivum* L. Cultivars

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Fifteen wheat genotypes were grown under water deficit to ascertain the role of osmotic adjustment (OA) and the concentration of benzoxazinones in sustaining grain yield. A positive correlation between osmotic adjustment capacity and yield was observed in wheat genotypes cultivated under field conditions. The weight gain of plants exposed to drought was in agreement with the OA values ($R^2 = 0.93$). However, when wheat plants were infested by cereal aphids, this correlation was not found. The benzoxazinones 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA) and 2,4-dihydroxy-7-methoxy-1,4 benzoxazin-3-one (DIMBOA) are defensive secondary metabolites present in wheat and others cereals. The content of these compounds varied in wheat genotypes and increased with drought and aphid infestation. A positive correlation between weight gain of irrigated-infested plants and drought-infested plants and the contents of benzoxazinones was observed. These results suggest that plants with better OA capacity and high benzoxazinone content should have better field yields.

Key words: Wheat, Osmotic Adjustment, Benzoxazinones, Crop Yield

Introduction

Under water stress some plants accumulate solutes in the cells to maintain cell turgidity, which is important for growth. This process is called osmotic adjustment (OA). Solute accumulation decreases the water potential of leaves, stem and roots allowing the plant to absorb water from the soil at lower water potentials. OA has been observed in *Sorghum vulgare* L. (Santamaría and Ludlow, 1990), *Oryza sativa* L. (Lilley and Ludlow, 1998), *Solanum tuberosum* L. (Bussis and Heineke, 1998), and *Triticum aestivum* L. (Moinuddin and Khanna-Chopra, 2004; Morgan, 1995).

It has been postulated that cultivars with higher OA capacity produce higher yields than those with lower OA capacity (Abebe *et al.*, 2003; El Hafid *et al.*, 1998). However, other studies with the same genotypes did not show such a correlation (Munns, 1988; Ludlow and Muchow, 1998; Blum *et al.*, 1999). Probably, the presence of other unaccounted factors could explain the differences obtained in similar studies. In this investigation we considered the presence of two genotype characteristics of the wheat plant such as the presence of chemicals that are important resistance factors against diseases or pests and the osmotic adjustment capacity of plants. Benzoxazinones are metabolites present in several species of higher plants (Pratt *et al.*, 1995; Bravo and Copaja, 2002; Bravo *et al.*, 2004a), particularly in cereals of agricultural importance such as *Zea mays*, *Triticum* sp. and *Secale cereale* (Niemeyer, 1988; Sicker and Schulz, 2002). These compounds are important factors of host plant resistance against microbial diseases and insects and act as allelochemicals (Argandoña *et al.*, 1981; 1983; Barnes *et al.,* 1987; Pérez, 1990; Friebe *et al.*, 1997; Bravo *et al.,* 2004b). Several authors showed that a decrease in pathogen or pest injury proved to be positively correlated with an increase in benzoxazinones content in maize, wheat and rye. Furthermore, the contents of these compounds increase when plants are under water stress (Richardson and Bacon, 1993). The hypothesis of this work was: Wheat cultivars with high content of benzoxazinones and high osmotic adjustment capacity have higher yield than cultivars without these characteristics cultivated under drought and aphid-infested conditions.

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Experimental

Chemicals

2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) and 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA) standards were isolated from extracts of maize (*Zea mays* L. cv. T-128) and rye (*Secale cereale* L. cv. Tetra), respectively, as previously described (Queirolo *et al.*, 1983; Lyons *et al.*, 1988).

Field trials

Trials were done in the Antumapu Experimental Field of Agronomy Faculty of the University of Chile, Santiago, Chile (33° 40′ S; 70° 38′ O).

Fifteen *Triticum aestivum* genotypes were grown in the field in two independent trials: under irrigated and under drought conditions. In the first trial, plants were irrigated when the soil moisture status at 50 cm depth decreased to 50%. Plants in the second trial received only an initial irrigation and 62.5 mm of rain. Seeds were obtained from the International Center of Corn and Wheat Improvement (CIMMYT, Mexico), International Center for Agriculture in Arid Zones Research (ICARDA, Syria) and from the Instituto Nacional de Investigaciones Agropecuarias (INIA, Chile), and treated with Lorsban (chlorpyrifos) and Til (propiconazole) to control insects and fungi, respectively.

The sowing season was late winter (August). The seed dosage was 12 g m^{-2}. The date of harvest was early summer (December).

The values of grain yield (in kg ha^{-1}) were obtained as follows: plants from each parcel (1 m^2) were harvested and submitted to a stationary threshing machine and the total weight of grain was measured (TW). In order to consider only the dry weight (DW) of the seeds, the humidity content was determined. 250 fresh grains were weighed to determine the fresh weight (FW) and then dried at 90 °C to obtain the DW. The grain yield was expressed in kg ha^{-1}:

$$\text{grain yield} = \frac{(\text{DW/FW})\ (\text{TW})}{\text{m}^2} \times 10^4. \qquad (1)$$

Greenhouse assays

30 seeds of genotypes G-80, G-65 and G-86 were planted in pots (2000 mL) filled with the same soil used in the field trials and grown at (24 ± 2) °C under a 12 h photoperiod and irrigated until field capacity. After 12 d, 12 plants from each pot of similar size were chosen and the others were eliminated. Then, four groups of each genotype were separated. One group was irrigated every 4 d (control), a second group was similarly irrigated and infested with the aphid *Rhopalosiphum padi* (10 adults on each plant). A third group was not irrigated and the fourth group was not irrigated but infested with aphids. 7 d later the dry weight and the DIBOA and DIMBOA content of leaves of each genotype were determined.

The plant weight percentage was calculated as follows:

$$\text{plant weight (\%)} = \frac{(\text{control plant weight} - \text{treatment plant weight}) \times 100\%}{\text{control plant weight}}. \qquad (2)$$

Osmotic adjustment determination

OA was determined as the difference in osmotic potential at full turgidity measured between irrigated and non-irrigated plants (group of plants irrigated only at the beginning of the experiment). Osmotic potential values were obtained *in situ* from the leaf material collected at noon-day. This material was frozen inside an Eppendorf tube with liquid nitrogen. Later the material was thawed and placed in a syringe (1 mL) and the cellular juice was obtained by mechanical pressure. An aliquot of 10 μL was used to saturate a disc of filter paper and the osmolality (mmol kg^{-1}) was measured with a Wescor 5520 osmometer (Wescor Inc., Utah, USA). The value was transformed to osmotic potential from the relationship: $\psi_s = C \cdot T \cdot R$, where C is the osmolality, T is the absolute temperature and R is the gas constant (0.00831 MPa mol^{-1} K^{-1}). The samples for determination of water parameters were collected in each experiment during the anthesis phenophase.

Chemical analysis

The contents of the two main hydroxamic acids found in wheat extracts (Zúñiga *et al.*, 1983), DIMBOA and DIBOA, were determined by high-perfomance liquid chromatography (HPLC). Thus, 0.1 g fresh leaves were triturated with a mortar and a pestle and 3 × 0.5 mL deionized water. The aqueous extracts were left at room temperature for 30 min, acidified to pH 3.0 with 0.1 N H_3PO_4 and centrifuged at 7000 × *g* for 15 min. The volume of supernatant was adjusted to 2.0 mL with deionized water and a 50 μL aliquot was directly injected into the HPLC instrument equipped with a Lichrosfer RP-100 C_{18} column (250 × 4 mm, particle size 5.0 μm). Chromatographic analysis was performed by isocratic elution with a 30:70 mixture of methanol and water (pH 3.0, 0.1 N H_3PO_4). Flow rate was 1.5 mL min^{-1} and detection was carried out at 263 nm (UV absorbance detector). The detection limit was 1.0 μmol kg^{-1} FW. All experiments were done with five replicates. Quantification was done from calibration curves obtained by linear regression.

The yield of degradation products of DIBOA and DIMBOA is a function of pH value, temperature, composition of the reaction medium and time (Bravo and Niemeyer, 1985; Smissman *et al.*, 1972; Grambow *et al.,* 1986). DIMBOA and DIBOA decompose in solution mainly to MBOA and BOA, respectively. The degradation of compounds under the conditions that we used to extract and to quantify the compounds was lower than 0.1 mmol kg^{-1} FW.

Statistical analysis

Assays were done according to a randomized block pattern with two repetitions. The experimental design of each field trial was random blocks with 4 repetitions. The experimental unit was a parcel of 4 by 1.6 m (8 rows separated by 0.2 m). The field results were analyzed using MSTAT-C (Michigan State University, 1988). The differences between genotypes and assays were analyzed by ANDEVA and Duncan's test. The association between variables was analyzed by regression.

Results and Discussion

Relationship between osmotic adjustment and grain yield of wheat genotypes under irrigated and drought conditions in field trials

The active accumulation of solutes in plant tissues as a response to water stress is called osmotic adjustment. This characteristic allows some plants to maintain their turgidity and physiological activity when the soil water is limited. Fig. 1 shows a correlation between OA values and grain yield of *Triticum aestivum* L. of fifteen genotypes of *Triticum aestivum* L. The OA values were in the range of 0.1–0.6 MPa. Genotype G-80 had the highest, genotype G-86 the lowest and genotype G-65 had an intermediate OA value. The dispersion observed (R = 0.83) could be attributed to other factors involved in the grain yield, such as resistance

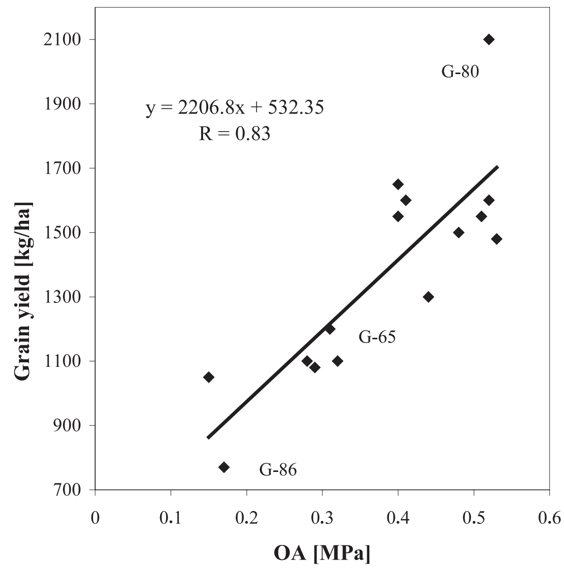

Fig. 1. Relationship between osmotic adjustment (OA) and grain yield of fifteen genotypes of *Triticum aestivum* L. cultivated under field conditions. Each point represents the mean of 4 samples of each genotype. Standard errors were lower than 14% and are omitted for simplicity.

to pathogen attack. However, we can conclude that grain yield should be positively related to the OA capacity of the different genotypes.

Effect of drought and aphid infestation on plant weight of genotypes with different osmotic adjustment capacity

Genotypes G-80, G-65 and G-86 that possessed high, intermediate and low OA capacity, respectively, were grown under greenhouse conditions. Table I shows the percentage of plant weight un-

Table I. Relationship between osmotic adjustment (OA) and percentage of weight gain of plants with respect to control (irrigated and non-infested) under drought (D), drought-aphid infestation (Di) and irrigated-aphid infestation (Ii) conditions.

Genotype	OA	Weight gain (%) of plants with respect to control		
		D	Di	Ii
G-80	0.49	91a	76b	85a
G-65	0.33	88a	54c	68d
G-86	0.18	80b	70d	78b

Each value corresponds to the mean of 6 samples. Means followed by different letters differ significantly (P < 0.05, LSD test). The weight gain of plants control (irrigated and without infestation) was assigned as 100%.

Table II. Effect of drought and aphid infestation on the benzoxazinone content (DIMBOA + DIBOA) in wheat genotypes.

	Benzoxazinones [mmol/kg FW]			
Genotype	I	Ii	D	Di
G-80	3.0a	3.3a	3.5c	4.7d
G-65	2.0b	2.2b	2.9a	2.6b
G-86	2.8a	3.1a	3.1a	3.8c

Each value corresponds to the mean of 6 samples. Means followed by different letters differ significantly ($P < 0.05$, LSD test).
I, irrigated plants; Ii, irrigated and infested plants; D, plants under drought conditions; Di, plants under drought and infested conditions.

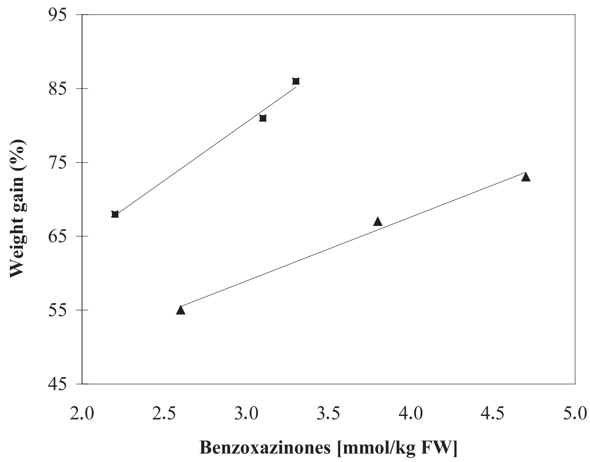

Fig. 2. Weight gain of three wheat genotypes that differ in benzoxazinone content (G-80, G-65 and G-86), cultivated under irrigated-infested (■) and drought-infested (▲) conditions. Each point is the mean of 4 samples ± s.e. and represents the percentage of weight gain of plants grown under irrigated and non-infested conditions.

der drought, drought-infested and irrigated-infested growing conditions. These values were calculated relative to the control (irrigated and non-infested). Plants exposed to drought and infestation (Di) showed the lowest weight. The weight gain of plants exposed only to drought (D) showed a positive correlation with the OA capacity of each genotype ($R^2 = 0.93$). These results support the hypothesis that the OA capacity accounts for the yield in cereals. However, when plants were infested with the aphid *Rhopalosiphum padi* this relationship was not observed. This may be related to the content of DIBOA and DIMBOA in the plants. It has been reported previously that these compounds are the main hydroxamic acids present in wheat and that they are toxic and repellent against aphids (Niemeyer, 1988; Sicker and Schulz, 2002). Also, their contents increase when plants are exposed to water stress (Richardson and Bacon, 1993) or attacked by aphids (Gianoli and Niemeyer, 1997). Therefore, the effects of drought and infestation on the contents of these benzoxazinones in genotypes G-80, G-65 and G-86 were evaluated. Table II shows the total (DIMBOA + DIBOA) content in the three genotypes cultivated under different watering and infestation conditions in a greenhouse. Water stress and infestation (Di) by *R. padi* increased the benzoxazinone content in all three genotypes. A significant increase was observed in genotypes G-80 and G-86. The influence of increased benzoxazinone contents on plant weight was evaluated by correlating the total content of benxozazinones and percentage of weight gain for plants under irrigated-infested and drought-infested conditions (Fig. 2). In both cases, the percentage of weight gain in respect to control plants increased with the content of benzoxazinones. The ability of benzoxazinones to repel insects is related to their concentration. Feeding assays have shown that DIMBOA has antifeedant effects at concentration as low as 1 mM, and at higher concentrations feeding is completely inhibited (Argandoña *et al.*, 1981). The relationship between the benzoxazinone levels in plants and resistance of the plant to insects, fungi and bacteria, detoxification of herbicides and allelopathic effects has been established in several papers (Bravo and Copaja, 2002; Argandoña *et al.,* 1981 1983; Pérez, 1990; Bravo *et al.*, 2004b). Contents of benzoxazinones in the three genotypes analyzed are in the range needed to produce defensive effects against *R. padi*. Consequently, the increase of plant weight should be in agreement with the increase of resistance of the plant against aphids. Therefore, our results suggest that plants with better OA capacity and high benzoxazinone content should have better field yields. These natural factors could be an alternative to the increasing use of pesticides.

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