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Carotenoid Compounds in Grapes and Their Relationship to Plant Water Status

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The aim of this work was to study the relationship between carotenoid contents in grapevine berries and plant water status. For this purpose, a black grapevine variety, *Vitis vinifera* L. cv. Touriga Nacional, was studied. The experiments were carried out in the same Douro vineyards, with plants of the same age, in two different water retention soils. A higher water retention capacity soil, soil A, and a lower water retention capacity soil, soil B, were both in a 1.2 m deep silt−loam schist-derived soil. The training system was the double cordon trained and spur pruned. A first range was nonirrigated (NI) and a second one was irrigated (I), 60% of evapotranspiration (ET_0). For soil B, a 30% of ET_0 treatment was also applied. The plant water status was estimated by predawn leaf water potential. The effects of plant water status on berry growth were studied by measurement of the berry weight and total soluble solids (°Brix). The carotenoid profile was quantitatively determined by high-performance liquid chromatography/diode array. Carotenoids determined were β-carotene, lutein, neoxanthin, violaxanthin, and luteoxanthin. The comparison between irrigated and nonirrigated grapes was followed from 2 weeks before veraison until the ripe stage. Results showed that at harvest time, berries exposed to the NI had a lower weight than those exposed to the irrigated treatment (60% of ET_0), 0.89 vs 1.36 g/berry and 0.94 vs 1.34 g/berry, for soils A and B, respectively. The irrigated treatment contributed to a higher sugar concentration in both soils. However, depending on the soil water retention capacity, the carotenoid contents were different in soils A and B. For soil A, the total carotenoid content was similar for both NI and I treatments. However, with regard to soil B, in irrigated treatment, levels of carotenoids were approximately 60% lower than those found for the NI. It seems to be possible to produce higher weight berries (with higher sugar levels) with similar carotenoid contents. On the other hand, soil characteristics had a larger influence than irrigation on the concentration of carotenoids in grapes, resulting in an important viticultural parameter to take into account in aroma precursor formation.

KEYWORDS: ***Vitis vinifera*; carotenoids; plant water status; irrigation; berry growth**

INTRODUCTION

The presence of carotenoids in grapes is well-documented, having been demonstrated that β-carotene and some xanthophylls (neoxanthin, flavoxanthin, and lutein) are abundant before veraison, and subsequently decreasing dramatically (*1*−*3*). Three other xanthophylls, namely, violaxanthin, luteoxanthin, and 5,6-epoxylutein, appear after veraison, when the sugar concentration reaches approximately 160 g/L (*3*). Cultivar, viticultural region, exposure to sunlight, and ripening stage all affect carotenoid concentrations in grapes (*4*−*7*). Carotenoids are known as precursors of C13−norisoprenoid compounds (*8*, *9*), which have been identified in grapes and wines and are known to be responsible for the typical aroma of some varieties.

The effect of irrigation on berry composition (°Brix, glucose, fructose, pH, organic acids, mineral elements, and phenolic content), color, and weight (g/berry) has been reported by many authors (*10*−*15*). It is reported that sugar content increases when irrigation is applied during the ripening stage, while irrigation during the early stages of berry development brings about an increase in grape yield together with a decrease in sugar concentration (*10*). Recent studies have indicated that weight (g/berry), °Brix, and glucose and fructose concentrations are significantly higher in Is than NIs (*11*, *12*). This fact could be related to a higher vegetative growth (I) resulting in higher pruning weights and a higher leaf area index, where irrigated vines are not exposed to heat stress as compared to nonirrigated

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Table 1. Definition of Soil Type, Irrigation Regime, and Climatic Conditions for Elemental Plots[a]

plot code	ANI	AI 60%	BNI	BI 60%	BI 30%
soil	soil type A	soil type A	soil type B	soil type B	soil type B
irrigation regime	NI	60% of ET_0	NI	60% of ET_0	30% of ET_0
training system	DCSP	DCSP	DCSP	DCSP	DCSP
vine spacing	2.2 m × 1.0 m	2.2 m × 1.0 m	2.2 m × 1.0 m	2.2 m × 1.0 m	2.2 m × 1.0 m
vines per hectare	4500	4500	4500	4500	
rainfall (mm) (June–Sept)	107.6	107.6	107.6	107.6	107.6
average temp (°C) (June–Sept)	23.5	23.5	23.5	23.5	23.5
ET_0 (mm) (June–Aug)	380	380	380	380	380
water applied (mm)	0	240	0	240	120

[a] Soil type A, high water retention capacity soil: initially escavated to 1.2 m depth with the base rock (schist) at approximately 0.8 m deep. The stoniness of this soil is approximately 40%. Soil type B, low water retention capacity soil: initially excavated to 1.2 m depth with the base rock (schist) at approximately 0.4 m deep. The stoniness of this soil is approximately 80%. DCSP, double cordon and spur pruned.

Table 2. Data Used for Carotenoid Identification in Grape Berries

		spectral data, λ_{max} (nm)						
pigment identified	tr (HPLC)	HPLC solvent			ethanol			source of pigment
neoxanthin	5.5	415	438	466	415	438	467	CaroteNature
violaxanthin	6.0	418	441	471	420	441	471	CaroteNature
luteoxanthin	6.2	400	422	448				
lutein	13.6	(422)	447	476	(421)	446	475	Sigma (alfalfa)
IS (β-apo-8′-carotenal)	18.8		460		464			Fluka
chlorophyll *a*	30.2		410					Aldrich (spinach)
β-carotene	32.4	(428)	454	482				Sigma

vines that suffer high temperatures (*12*). The organic acids (tartaric acid, malic acid, and citric acid) are present in high concentrations in green grapes and begin to decrease at veraison (*12*). The rate of decrease is greater for malic acid than for tartaric acid, and this difference is greatest in NIs (*12*). Medium or severe water deficits, occurring in the early stages of growth, have significant effects on cell size but not on cell division (*13*), while water deficit levels occurring during the period from veraison to maturity stimulate phenolic biosynthesis (*13*−*15*).

The aroma potential during ripening, caused by moderate irrigation, showed higher values for Is (*16*). The relationship between carotenoid contents in grapevine berries and plant water status has not yet been established. The aim of this present study was to examine the relationship between carotenoid content in grapevine berries and plant water status. For this purpose a black grapevine variety, *Vitis vinifera* L. cv. Touriga Nacional, was studied during the 2 weeks before veraison until full ripeness. The effects of plant water status on berry growth were studied by measuring the berry weight and °Brix, and the carotenoid profile was quantitatively determined by high-performance liquid chromatography (HPLC)/diode array.

MATERIALS AND METHODS

Plant Material. This experiment was conducted during 1 year (2002) with the variety *Vitis vinifera* L. cv. Touriga Nacional. Vines were 6 years old and were planted with 196-17 rootstock in the same vineyard plot in the Douro Superior subregion of northern Portugal.

Soils Types, Irrigation, and Sampling. Samples were taken from vines segregated into elemental plots (45 vines each) defined in terms of soil type and irrigation regime. The sampling period was from 2 weeks prior to veraison until full ripeness (19/07, 22/08, 4/09, and 19/09). All plots can be considered equal in terms of vine training system, plant density, and climatic conditions (**Table 1**). The ET_0 was estimated by the Penman−Monteith method as described by Allen et al. (*17*). The irrigation period was from 14/06 till 19/09. °Brix was measured using a refractometer LEICA-model 7530.

Extraction and Determination of Carotenoids. Grape Material. Approximately 50 g of fresh berries, of seeds, were homogenized using a Turrax homogenizer at 9500 rpm for 15 min. This procedure provided 40 g of sample that was spiked with 200 μL of internal standard and 170 mg/L of β-apo-8′-carotenal (Fluka, Portugal) (10810) and was diluted with 40 mL of water (18.3 MΩ/cm). Extraction was carried out with 40 mL of ether/hexane (1:1, v/v), HPLC grade (MERCK, Portugal), and agitated for 30 min. The extraction was repeated two more times with 20 mL of ether/hexane (30 min each). The final combined extract was concentrated to dryness (rotavapor) and resuspended in 1 mL of acetone/hexane (1:1, v/v) for HPLC determination. Light exposure was minimized during sample preparations in order to avoid photoisomerization.

HPLC. A Beckman model 126 quaternary solvent system, equipped with a System 32 Karat software and a 168 rapid-scanning, UV−visible photodiode array detector, was used. The absorption spectra were recorded between 270 and 550 nm.

Stationary Phase. Nova-Pack C18 60 Å 4 μm particles (3.9 × 300 mm), Waters.

Mobile Phase. Solvent A, ethyl acetate (Merck pure grade); solvent B, acetonitrile/water (9:1 v/v) (Merck pure grade and pure water); flow rate = 1 mL/min. The following gradient was employed, 0−31 min (0−60% A); 31−46 min (60% A); 46−51 min (60−100% A); 51−55 min (100% A); 55−60 min (100−0% A); 60−65 min (0% A). R_t values: neoxanthin (5.5 min), violaxanthin (6.0 min), luteoxanthin (6.2 min), lutein (13.6 min), unknown (27.5 min), chlorophyll (30.2 min), and β-carotene (32.4 min) (*18*).

Identification. Carotenoids were identified by comparison with commercially available standards, β-carotene (Sigma 95%, synthetic) (C-9750), lutein (Sigma 70%, from alfalfa) (X-6250), neoxanthin (0234.1) and violaxanthin (0259) from (CaroteNature GmbH from Switzerland), and chlorophyll a (Aldrich, from spinach) (25 825-3). Luteoxanthin was identified by comparison of retention time and UV−visible photodiode array spectra (**Table 2** and **Figure 1**).

Statistical Analysis. Principal component analysis (PCA) and cluster analysis (dendogram) were carried out using a XLSTAT-Pro version 6.1.8. The PCA method shows similarities between samples projected on a plane and makes it possible to determine which variables determine

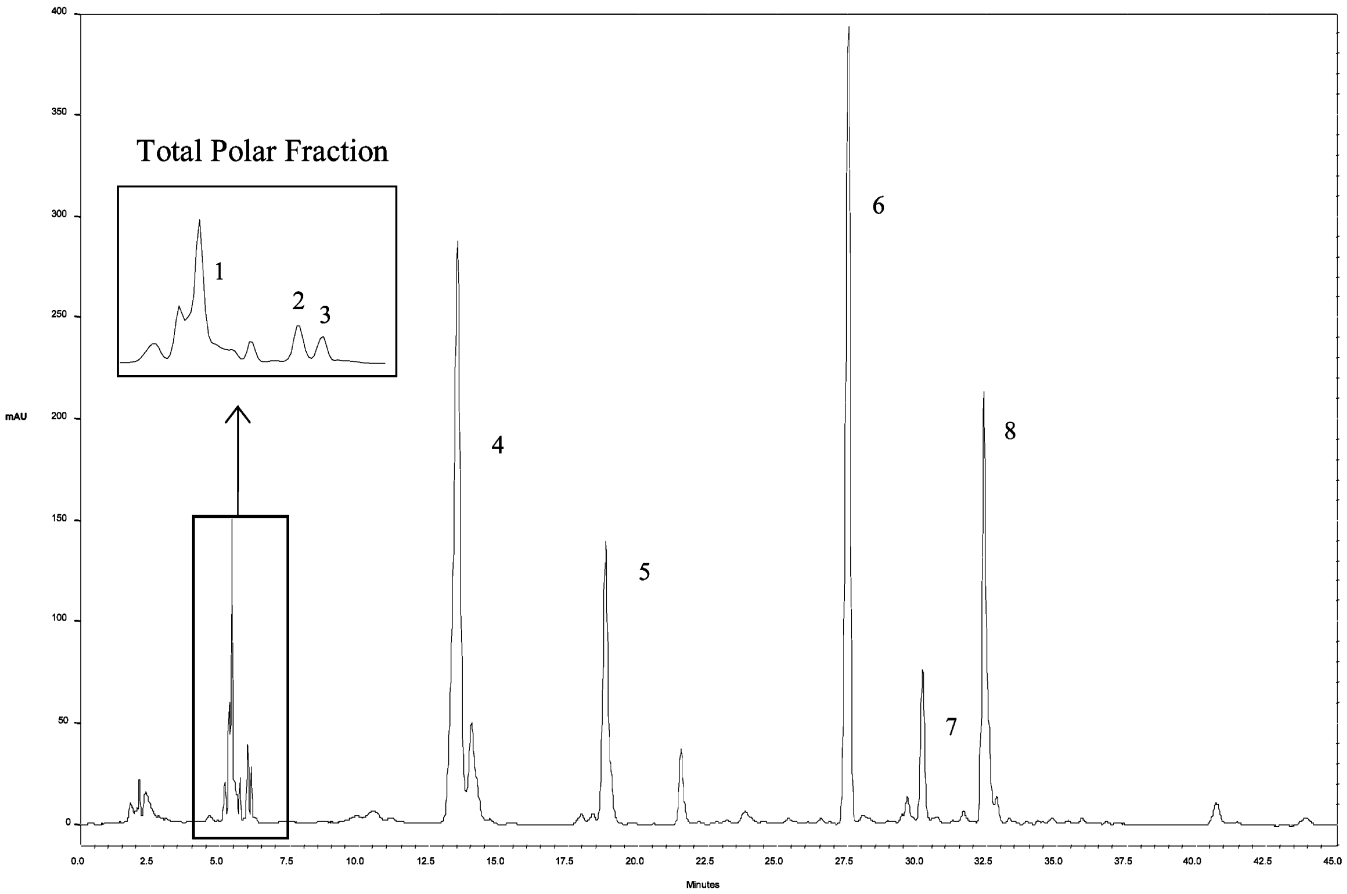

Figure 1. HPLC chromatogram of carotenoids of Touriga Nacional from BNI grapes at harvest time (19/09). DO = 447 nm. (1) Neoxanthin, (2) violaxanthin, (3) luteoxanthin, (4) lutein, (5) internal standard, (6) unknown, (7) chlorophyll *a*, and (8) β-carotene.

Table 3. Changes in Carotenoid Contents of the Grape Berries Harvested from Different Soils with and without Irrigation during Ripening[a]

plot code	neoxanthin	violaxanthin	luteoxanthin	lutein	chlorophyll *a*	carotene	weight/berry	°Brix
ANI_I	66	0	0	1183	327	1981	0.48	6
ANI_II	92	22	2	929	163	1363	0.88	15
ANI_III	70	23	1	571	90	918	0.90	17
ANI_IV	85	12	0	624	93	1046	0.89	17
AI 60%_I	115	0	0	1499	334	2153	0.56	5
AI 60%_II	71	16	2	548	94	764	1.56	18
AI 60%_III	65	23	2	519	80	746	1.52	22
AI 60%_IV	164	27	4	874	121	1204	1.36	25
BNI_I	65	0	0	1563	360	2018	0.50	6
BNI_II	49	6	1	514	89	739	0.94	16
BNI_III	63	7	2	452	67	677	0.95	19
BNI_IV	98	26	17	728	116	1102	0.94	19
BI 30%_I	33	0	0	881	198	1198	0.57	6
BI 30%_II	39	1	1	340	55	448	1.39	21
BI 30%_III	41	10	4	260	38	369	1.26	23
BI 30%_IV	32	6	1	152	21	238	1.02	24
BI 60%_I	44	0	0	855	194	999	0.54	5
BI 60%_II	94	4	1	615	110	721	1.42	19
BI 60%_III	84	17	0	629	93	762	1.20	23
BI 60%_IV	57	9	0	282	40	356	1.34	26

[a] I, sample data for 19/07; II, sample data for 22/08; III, sample data for 04/09; IV, sample data for 19/09. Carotenoid concentration is expressed in μg/kg of berry. Neoxanthin, violaxanthin, and luteoxanthin are expressed in equivalents of lutein. Berry weight is in grams.

these similarities and in what way. The dendogram method shows correlations by clusters diagrams.

RESULTS AND DISCUSSION

The effect of irrigation on changes in carotenoid contents for soil A and soil B is shown in **Table 3**. The comparison was made from 2 weeks before veraison, during ripening, and at harvest time (19/07, 22/08, 4/09, and 19/09). Carotenoids analyzed were β-carotene, lutein, neoxanthin, violaxanthin, and luteoxanthin. Chlorophyll *a* was also considered due to its major impact during grape ripening, as an indicator of maturation. Results showed that from all plots and experimentation, carotenoid content decreased during ripening with a concomitant

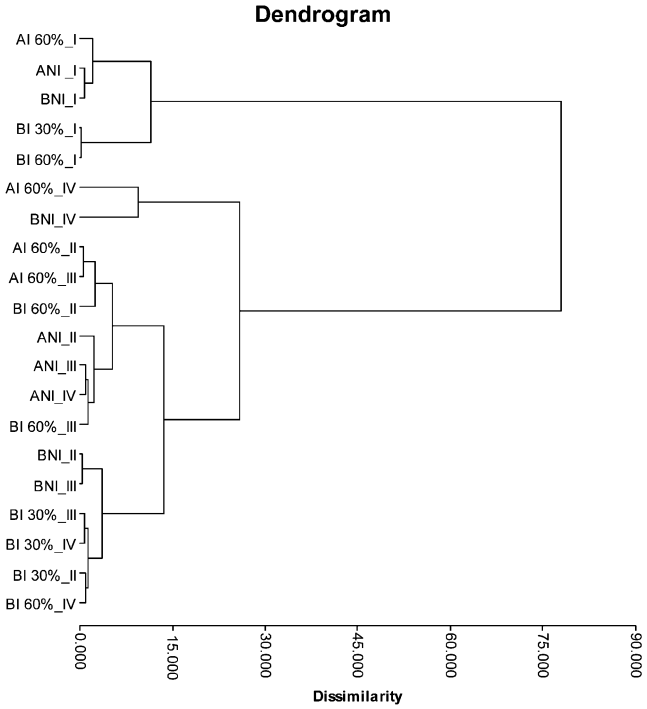

Figure 2. Dendogram of the carotenoid contents with different regimes of irrigation in the two analyzed soils.

increase of °Brix. The largest percentage decreases were observed for chlorophyll *a*, lutein, and β-carotene of 64−89, 42−83, and 44−80%, respectively. Violaxanthin and luteoxanthin appeared only from 22/08 and seem to have slightly increased during this period (**Table 3**). These results are in agreement with previous work where the presence of carotenoids in grape berries demonstrated that β-carotene and several xanthophylls are abundant before veraison, with decreasing levels during ripening. These decreases were less prominent during the maturation (*3*).

Berry growth was studied by measurement of the berry weight and °Brix (**Table 3**). Is contributed to an increase of berry size and to higher sugar concentrations in both soils (A and B). The higher sugar accumulation noted in irrigated vines may be related to their higher photosynthetic activity as compared to water-stressed vines, prolonging the period of photosynthetic activity by slowing leaf senescence. This effect can contribute to a lower rate of sugar transport to the berries in nonirrigated vines (*11*). Furthermore, higher vegetative growth (I) results in higher pruning weights and higher leaf area index where irrigated vines are not exposed to heat stress as compared to nonirrigated vines, which suffer high temperatures (*12*).

Figure 2 shows the degree of correlation of carotenoid contents with different regimes of irrigation in the two different soils, while **Figure 3** gives the factors scores (factor score plot 1-2 accounts for 83% of total variance) from the principal components study carried out with data from **Table 3**. From a detailed study of these figures, it can be concluded that (i) for the first stage of maturation (I_19/07) two different groups can be seen, a first group (ANI, AI 60%, and BNI) and a second group (BI 30% and BI 60%), that follow different patterns of variation. Carotenoids found in nonirrigated and irrigated grapes in the higher water retention capacity soil (ANI and AI 60%) were similar. Because carotenoids are in much higher levels in skins than pulp (*17*), this nonvariation could be explained by the fact of observed increases in pulp−skin ratios with Is. Carotenoids found in the nonirrigated grapes in the lower water

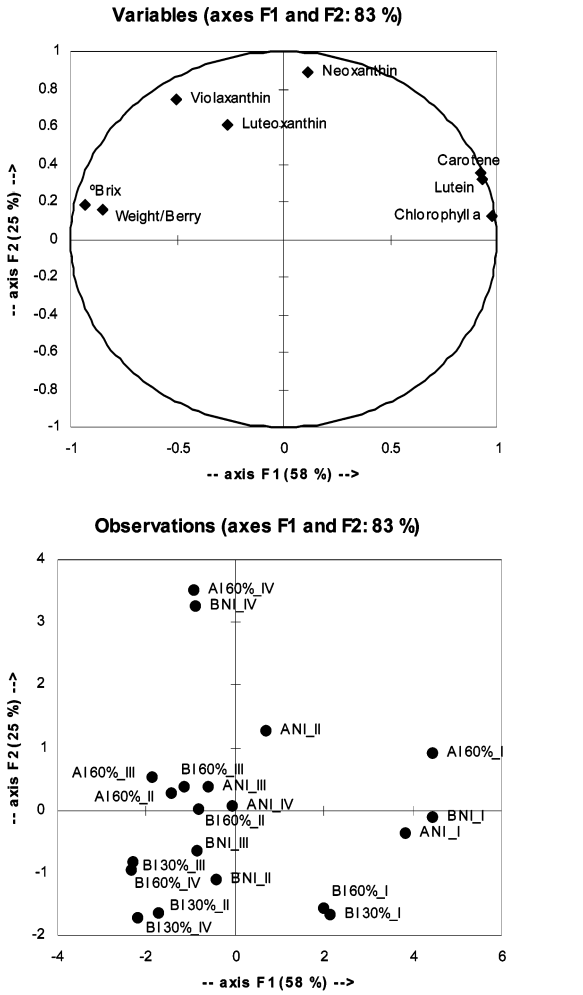

Figure 3. Principal components diagram of the carotenoid contents with different regimes of irrigation in the two analyzed soils. Factor score plot 1-2. Components 1 and 2 account for 83% of the total variance.

retention capacity soil (BNI) are close to those found in the higher water retention capacity soil (soil A). However, irrigation, in the lower water retention capacity soil (soil B), leads to a lower carotenoid concentration in the grapes. Nevertheless, different levels of irrigation, 30% and 60% of ET_0, had similar values of carotenoids for BI 30% and BI 60%, respectively. Carotenoid contents in irrigated grapes in the lower water retention soil (BI 60%) were approximately 61 and 68% lower for lutein and β-carotene, respectively, than that of the nonirrigated grapes (BNI).

(ii) For the last stage of maturation (IV_19/09), AI 60% and BNI are well-grouped, indicating that carotenoid levels were similar for a nonirrigated lower water retention capacity soil (BNI) and an irrigated higher water retention capacity soil (AI 60%).

(iii) The other plots, which all exhibit different behaviors, are different from the others. For (II_22/08 and III_4/09) stages, Is, in soil B, are well-grouped, with the exception of the 60% of ET_0 treatment. One these dates carotenoid decreasing, during ripeness, slowly down for this treatment. For soil A, the NI and I follows an identical variation in these stages of ripeness.

°Brix and berry weight are well-correlated. The correlation coefficient at the level of significance $\alpha = 0.050$ (two-tailed test) is 0.853. All carotenoids analyzed are well-correlated with °Brix (correlation values higher than −0.6 reach −0.868 for

chlorophyll *a*) with the exception of neoxanthin, violaxanthin, and luteoxanthin, which have different behaviors. Although °Brix and berry weight increases were concomitant with I, no direct effect could be established between these parameters and carotenoid contents in grapes. Soil characteristics and water retention capacity affect canopy density and consequently bunch exposure to sunlight. Light has probably the largest effect on carotenoids content.

CONCLUSION

Soil and water retention capacity affect carotenoid contents in grapes. I seems to contribute to lower carotenoid levels in grapes, when vines are planted in a lower water retention capacity soil. This decrease was similar for both 30 and 60% of ET_0. However, in a higher water retention capacity soil, I seems to have no effect in carotenoid contents when compared with NI.

It seems possible to produce grapes with higher weight and higher sugar levels together with similar carotenoid contents in an irrigated higher water retention capacity soil. It is reasonable to establish the possibility of improving wine production together with the eventual presence of substances with high aroma impact, knowing that carotenoids are precursors of several of these aroma compounds. On the other hand, soil characteristics had a larger influence than irrigation on the concentration of carotenoids in grapes, resulting in an important viticultural parameter to take into account in aroma precursor formation. To gather more information concerning other viticultural parameters that might help to better understand the results of the present work, ongoing research is under development.

ABBREVIATIONS USED

NI, nonirrigated treatment; I, irrigated treatment; ET_0, potential evapotranspiration; 30% of ET_0, 30% of evapotranspiration; 60% of ET_0, 60% of evapotranspiration; °Brix, total soluble solids; ANI, nonirrigated treatment in soil A; AI 60%, irrigated treatment at 60% of evapotranspiration in soil A; BNI, nonirrigated treatment in soil B; BI 60%, irrigated treatment at 60% of evapotranspiration in soil B; BI 30%, irrigated treatment at 30% of evapotranspiration in soil B.

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