

Leaf petiole mineral and fruit heavy metals content of different grape cultivars grown under arid environments and irrigated with treated domestic wastewater

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Abstract. This study was carried out during 2008 and 2009 years at the Research and Agricultural Experimental Station, King Saud University, Saudi Arabia, in order to study the comparative effect of some grapevine cultivars grown under arid environments and irrigated with treated domestic wastewater on leaf petiole mineral and fruit heavy metals content. The data indicated that the Thompson Seedless, Perlette, Des El-Anz and White Shatta had similar and lower leaf N than all other cultivars. Flame seedless had lower P. Flame seedless, Perlette and Thompson seedless had similar and lower leaf K than all other cultivars. Flame seedless and Thompson Seedless had similar and lower Ca₂ than all other cultivars. Thompson Seedless had higher leaf Mg than white Shatta, Perlette, Des El-Anz, Taify, Soltany and Red Glop cultivars. In addition, Taify had significantly higher Fe than King Ruby, Des El-Anz and Soltany cultivars. Flame Seedless had significantly higher Zn and Mn than all other cultivars. The Des El-Anz, Taify, Kamaly, Soltany and White Shatta cultivars had similar and higher fruit Cu than Thompson Seedless, Flame Seedless, Red Glop and King Ruby. Moreover, Des El-Anz had higher fruit Cd than all other cultivars, except Taify, Soltany and Halawany. Fruit NO₃ of Flame Seedless and Kamaly was similar and higher than all other cultivars, except Soltany and King Ruby. Kamaly, Flame Seedless and Soltany had similar and higher fruit NO₂ than King Ruby, Red Glop, Perlette and Thompson Seedless.

Key Words: grapevines, leaf petiole mineral, wastewater, arid environments, fruit heavy metals, comparative studies.

Introduction. Grapevine (*Vitis vinifera* L.) is one of the world's largest fruit crops. Its area has reached 7.437.141 hectares producing 66.935.199 tons worldwide, while, it reported as 11.675 hectares producing 144.430 tons in Saudi Arabia according to FAO (2010). In Riyadh, beside local grape varieties already grown in this region, cultivation of new grape varieties has been given great concern. However, this part of Saudi Arabia is characterized with aridity, coarse-textured soil with high CaCO₃ levels, lower organic matter content and native available Zn and Fe (Modaihsh 1992) and treated wastewater is used for irrigation. Therefore, many nutritional and fertility problems arise seriously in such condition, as well as low productivity and poor fruit quality is expected.

For sustainable viticulture, it is important to know the interaction influences of cultivars, soil characters, climatic conditions, and irrigation water type on vine productivity (Keller et al 2001)

The reuse of treated effluent water for irrigation provides an effective and rich source of nutrients (Bastian & Ryan 1986). However, it may pose detrimental effect on nutrients release to the environment (Paranychianakis et al 2006) and heavy metals

content of the soil (Singh et al 2004; Mapanda et al 2005). Furthermore, this type of water contains high salts levels (especially Na and Cl) and high electrical conductivity which are associated with detrimental effects on plant growth due to osmotic stress, ion toxicity and nutritional disturbances (Cheeseman 1988). Several studies conducted in many arid and semi-arid regions of the world have shown that wastewater can be used to irrigate certain crops without a significant loss in yield or quality. Many researchers have observed that some plants species are endemic to metalliferous soils and can tolerate greater than usual amounts of heavy metals or other toxic compounds (Blaylock & Huang 2000; Raskin & Ensley 2000).

The uptake of nutrients from the soil depends on different factors namely; their soluble content in it, soil pH, plant growth stage, plant species and types of soil and fertilizers (Sharma et al 2006). Plant species have a variety of capacities in removing and accumulating heavy metals, so there are reports indicating that some species may accumulate specific heavy metals, causing a serious health risk to human (Wenzel & Jackwer 1999).

The success of viticulture is based on three columns. These are: growing site, cultivars and growing technology. Out of these three factors growing site is dominant. Its agro-ecologic potential can be altered by the viticulture is by choosing the proper variety and trellising system. Local varieties are more resistant and are more suitable for extensive growing conditions. Their yield is sometimes lower than that of new cultivated ones, but their fruit quality is superb. Some new varieties have been recently introduced to Saudi Arabia and were planted in Riyadh region. Since those newly introduced cultivars were not previously tested for their growth and productivity under local conditions, some of them were selected for the evaluation of their performance which would be helpful to grape growers when deciding which cultivar to plant.

To obtain optimum growth and fruit quality, all nutrients must be present in sufficient concentrations. Therefore, accurate diagnosis of the grapevines nutritional status is essential before one can give sound advice on fertilizer treatment. There have been many reports concerning mineral uptake and its distribution in grapevines. El-Sharkawy (1995) found that there were significant differences between grapevine cultivars and their leaf petioles mineral content. Also, Fardossi et al (1995) confirmed that petioles leaf mineral concentrations could be influenced by grape cultivars.

This study therefore aimed to evaluate the leaf petioles mineral and fruit heavy metals content of some grape cultivars grown under the environmental conditions and treated domestic wastewater irrigation in Riyadh region.

Material and Method. The present investigation was conducted on different grapevines cultivars during the years 2008 and 2009 at the Research and Agricultural Experimental Station in Dirab, King Saud University, Riyadh, Saudi Arabia. The soil of the experimental orchard was sandy loam saline calcareous with 7.5–7.8 pH, 6.62–6.86 E.C., 33.7% CaCO₃ and 0.36% organic matter. The chemical properties of the treated domestic wastewater (irrigation water) are presented in Table 1.

During the winter time, a mixture of organic manure, potassium sulfate (48% K₂O), calcium mono super phosphate (15.5% P₂O₅) and ammonium sulfate (21% N) at the rates of 10 kg, 100 g, 150 g and 70 g/vine, respectively were annually ditched in the vineyard soil at a depth of 20–25 cm from the soil surface and 30 cm far from the vine trunk. In the meantime, 100 g ammonium sulphate, 100 g potassium sulphate, and 150 g calcium super phosphate/vine were also added, during each growing season. Vines were annually irrigated with treated domestic wastewater by flooding irrigation system.

Table 1

Chemical properties of the treated domestic wastewater (irrigation water)

Properties	value	properties	value
EC ds/m	2.58	B **	1.38
pH	6.99	Mn ***	15.1
Na ⁺ *	11.34	Cr ***	90.6
K ⁺ *	0.39	Co ***	4.9
Ca ⁺⁺ *	9.51	Ni ***	109
Mg ⁺⁺ *	5.25	Cu ***	291
HCO ₃ ⁻ *	3.72	Zn ***	204
SO ₄ ⁻⁻ *	17.62	As ***	1.64
CL ⁻ *	11.26	Cd ***	1.73
NO ₃ ⁻ **	9.04	Pd ***	15.8

* mEq/L ** ppm *** ppb

Eleven local and newly planted vine cultivars were used. The local cultivars were: Kamali, Soltany, Taify, White Shatta, Des El-Anz and Halawany; while newly cultivated ones were: Red Glop, Perlette, Flame Seedless, King Ruby and Thompson Seedless. Kamaly, Soltany, Taify, White Shatta, Des El-Anz, Halawani, Red Glop, Flame Seedless and King Ruby were cordon-spur-pruned, whereas Thompson Seedless and Perlette were cane-pruned. All vines were pruned to about 40-45 buds. Ten years old seedling grapevines were planted at 1.5 × 3 m spacing were selected uniform as possible in vigor from all cultivars with four replicates (2 vines / replicate) for each cultivar.

Vines nutritional status was studied by the determination of leaf petiole and fruit mineral content. During both seasons, leaf and fruit samples were collected from each replicate, for all cultivars. Leaf samples were selected carefully from the first fully mature leaves from the tips of growing shoots, while the fruit samples were taken at harvest time. The samples were brought directly to the Pomology Laboratory of the College of Food and Agricultural Sciences, King Saud University. The leaf petioles were separated from the blades and, petioles and fruit samples were then washed several times with tap and distilled water to remove any spray residues and other deposits. Thereafter, petioles and fruits samples were weighed then dried to a constant weight in a drying oven at 60-70°C. The dried samples were weighed and grounded in a stainless steel rotary knife mill to 20 mesh size and 0.3 gm of each sample (leaf petioles and fruits) was digested with H₂O₂ and H₂SO₄ according to Evanhuis & De Waard (1980). Suitable aliquots were taken for determination of nitrogen, phosphorus, potassium, calcium, magnesium, sodium, iron, manganese, zinc and copper concentration in leaf petioles and NO₃, NO₂, Cd, Pb and Ni in the fruits. N was determined by the Kjeldahl method. P was determined by ascorbic acid method as previously mentioned by Murphy & Riley (1962). Potassium and sodium content was measured by Coring 400 Flame photometer. The levels of Ca, Mg, Fe, Zn, Mn, Cu, Pb, Cd and Ni contents were measured by Perkin Elmer 305BA atomic Absorption Spectrophotometer. The concentrations of N, P, K, Ca and Mg were expressed as percent (%), while Fe, Mn, Zn, and Cu were expressed as parts per million (ppm); fruit Pb, Cd and Ni as mg/Kg on dry weight basis. Fruit nitrate (NO₃) and nitrite (NO₂) contents were measured according to Chapman & Pratt (1961) and expressed as mg Kg⁻¹ dry weight.

The study was a randomized complete block design (RCBD) with 11 cultivars and four replicates for each cultivar (2 vines / replicate). All obtained data were tested for cultivars and years effects on analyzed parameters by the general linear model (GLM) and two-way analysis of variance (ANOVA) technique as a combined analysis (over two years). Means of the effect of cultivars (first factor) and years (second factor) and their

interactions were separated and compared using the Honest Significant Differences (HSD) at 0.05 level of significance according to Snedecor & Cochran (1989). The statistical analysis was performed using SAS (Statistical Analysis System) version 8.1(1988).

Results. The data representing the leaf petioles nitrogen, phosphorus and potassium content are presented in Table 2. Regardless of the two years, leaf petioles of Thompson Seedless, Perlette, Des El-Anz and White Shatta cultivars had similar nitrogen content and significantly lower than Flame Seedless, King Ruby, Soltany, Halawany and Red Glop, which did not significantly differ from each other. Likewise, leaf petioles of Taify and Kamally cultivars had similar nitrogen content and lower than King Ruby, Soltany, Halawany and Red Glop. However, White Shatta, Perlette, Des El-Anz, Taify, Kamaly and Thompson seedless had similar nitrogen content. Regarding, the leaf petioles phosphorus content, Flame seedless had significantly the lowest phosphorus content as compared to all other cultivars (except Taify, Des El-Anz and Perlette), whereas, all other cultivars did not significantly differ in their petioles phosphorus content. As for, the potassium content, the data also showed that potassium content in leaf petioles of Perlette, Flame seedless and Thompson seedless cultivars was similar and significantly lower than all other cultivars. Furthermore, leaf petioles of Red Glop and Kamaly had similar potassium content which was significantly higher than Des El-Anz and White Shatta. However, the Halawany, Soltany, Kamaly, Taify, King Ruby and Red Glop cultivars did not significantly differ from each other. A significant interaction effect between cultivars and years on petioles nitrogen, phosphorus and potassium content was recorded for all studied cultivars.

Table 2

Leaf petiole N, P and K percent of the different grape cultivars
in 2008 and 2009 years

Grape cultivars	N			P			K		
	2008	2009	Ave.	2008	2009	Ave.	2008	2009	Ave.
Thompson Seedless	0.75	1.24	1.00	0.34	0.42	0.38	1.8	2.4	2.1
Flame Seedless	1.25	1.48	1.37	0.17	0.16	0.17	1.9	2.1	2.0
Red Glop	1.40	1.78	1.59	0.40	0.37	0.39	2.9	3.3	3.1
Perlette	0.77	1.41	1.09	0.27	0.30	0.29	1.7	2.4	2.1
King Ruby	1.39	1.66	1.53	0.36	0.42	0.39	2.7	3.1	2.9
Des El-Anz	1.00	1.13	1.07	0.26	0.24	0.25	2.2	3.0	2.6
Taify	0.85	1.42	1.14	0.26	0.27	0.27	2.4	3.1	2.8
Kamaly	0.87	1.38	1.13	0.27	0.34	0.31	2.9	3.3	3.1
Soltany	1.18	1.66	1.42	0.29	0.35	0.32	2.6	3.1	2.9
White Shatta	0.85	1.33	1.03	0.34	0.32	0.33	2.2	2.9	2.6
Halawany	1.11	1.71	1.41	0.35	0.32	0.34	2.7	2.9	2.8
HSD 0.05	0.38		0.27	0.20		0.14	0.8		0.5

The data concerning the calcium, magnesium and sodium concentration in the leaf petioles of the experimental cultivars are shown in Table 3. Regardless of the two years, calcium content in Flame seedless and Thompson Seedless was similar and was lower than all other studied cultivars (except Des El-Anz and Halawany). In addition, King Ruby had significantly higher calcium than Des El-Anz, whereas, no significant differences were found among the Red Glop, Perlette, Taify, Kamaly, Soltany, King Ruby, Halawany and White Shatta cultivars. As for leaf petioles magnesium content, the

data showed that Thompson Seedless had significantly higher magnesium than White Shatta, Perlette, Des El-Anz, Taify, Soltany and Red Glop, which were similar in their leaf petioles magnesium content. No significant differences were found among the Flame seedless, Halawany, King Ruby, Kamaly and Thompson seedless cultivars. Regarding the leaf petioles sodium content, the data showed that sodium content in the leaf petioles of Red Glop, Perlette and King Ruby cultivars was similar and higher than that of Halawany, Flame Seedless, Taify, Kamaly, Soltany, White Shatta and Thompson Seedless, which did not differ from each other. Furthermore, no significant differences were found between Des El-Anz, Red Glop, Perlette and King Ruby. A significant interaction effect between cultivars and years on leaf petioles calcium and magnesium content was recorded for all studied cultivars.

Table 3

Leaf petiole Ca, Mg and Na percent of the different grape cultivars in 2008 and 2009 years

Grape cultivars	Ca			Mg			Na		
	2008	2009	Ave.	2008	2009	Ave.	2008	2009	Ave.
Thompson Seedless	1.5	1.7	1.6	0.72	0.77	0.75	0.53	0.46	0.50
Flame Seedless	1.5	1.9	1.7	0.60	0.66	0.63	0.45	0.49	0.47
Red Glop	2.3	2.7	2.5	0.56	0.60	0.58	0.64	0.66	0.65
Perlette	2.2	2.4	2.3	0.59	0.55	0.57	0.63	0.67	0.65
King Ruby	2.6	2.8	2.7	0.60	0.66	0.63	0.65	0.67	0.66
Des El-Anz	1.9	2.3	2.1	0.54	0.61	0.58	0.54	0.60	0.57
Taify	2.0	2.6	2.3	0.61	0.59	0.60	0.46	0.46	0.46
Kamaly	2.2	2.6	2.4	0.66	0.68	0.67	0.46	0.48	0.47
Soltany	2.1	2.8	2.5	0.60	0.62	0.61	0.53	0.45	0.49
White Shatta	2.1	2.4	2.3	0.52	0.60	0.56	0.45	0.47	0.46
Halawany	1.9	2.4	2.2	0.58	0.65	0.62	0.47	0.53	0.50
HSD 0.05		0.9	0.6		0.21	0.14		NS	0.15

The data concerning the iron, zinc, and manganese content in the leaf petioles of the studied vine cultivars are listed in Table 4. The obtained results revealed that the Taify cultivar had significantly iron concentration than the King Ruby, Des El-Anz and Soltany cultivars, which had similar iron content. Likewise, Perlette, Kamaly and Flame Seedless were similar and had higher iron concentration than Des El-Anz and King Ruby. However, no significant differences were found between Thompson Seedless, Halawany, Red Glop and White Shatta cultivars in their leaf petioles iron content. As for the leaf petioles zinc and manganese content, Flame Seedless had significantly the highest zinc and manganese concentrations as compared to all other cultivars, except White Shatta and Soltany. The two latter cutivars had were similar in their leaf petioles zinc content and were higher than Taify, King Ruby and Perlette in their leaf zinc and manganese and, than Red Glop and Thompson Seedless in their leaf zinc only. A significant interaction effect between cultivars and years on leaf petioles iron, zinc and manganese content was recorded for all studied cultivars.

The data representing the copper, cadmium lead and nickel content in the fruit of the studied grape cultivars are listed in Tables 5-6. With regard to the fruit copper content, the Des El-Anz, Taify, Kamaly, Soltany and White Shatta cultivars had similar and higher copper content than Thompson Seedless, Flame Seedless, Red Glop and King Ruby, which did not differ from each other in their copper content. Furthermore, no significant difference in copper content was found between Halawany, Des El-Anz, Taify, Kamaly, Soltany, White Shatta and Perlette. In the main time, Des El-Anz had

significantly the highest fruit cadmium concentration in comparison with all other studied cultivars, except Taify, Soltany and Halawany. However, no significant differences were found among the Thompson Seedless, Flame Seedless, Red Glop, Perlette, King Ruby, White Shatta and Kamaly cultivars in their fruit cadmium content. As for fruit lead and nickel content, the data showed that all cultivars had significantly similar lead and nickel concentrations. Moreover, a significant interaction effect between cultivars and years on fruit cadmium content was recorded for all studied cultivars.

Table 4

Leaf petiole Fe, Zn and Mn content (ppm) of the different grape cultivars in 2008 and 2009 years

Grape cultivars	Fe			Zn			Mn		
	2008	2009	Ave.	2008	2009	Ave.	2008	2009	Ave.
Thompson Seedless	96	92	94	24	34	29	41	39	40
Flame Seedless	96	98	97	34	42	38	60	53	57
Red Glop	81	102	92	23	21	22	42	36	39
Perlette	94	100	97	26	27	27	28	34	31
King Ruby	75	99	87	21	27	24	25	30	28
Des El-Anz	78	97	88	22	42	32	44	40	42
Taify	97	101	99	24	30	27	30	42	36
Kamaly	94	98	96	28	36	32	34	45	40
Soltany	92	90	91	32	37	35	47	52	50
White Shatta	98	92	95	33	37	35	46	56	51
Halawany	89	99	94	28	30	29	42	37	40
HSD 0.05	12		8	9		6	21		14

Table 5

Fruit Cu, Cd and Pb content (mg kg^{-1}) of the different grape cultivars in 2008 and 2009 years

Grape cultivars	Cu			Cd			Pb		
	2008	2009	Ave.	2008	2009	Ave.	2008	2009	Ave.
Thompson Seedless	6	6	6	0.19	0.21	0.20	0.024	0.022	0.023
Flame Seedless	7	7	7	0.17	0.18	0.18	0.026	0.034	0.030
Red Glop	6	5	6	0.19	0.22	0.21	0.020	0.024	0.022
Perlette	9	10	10	0.26	0.16	0.21	0.024	0.034	0.029
King Ruby	7	7	7	0.28	0.17	0.23	0.028	0.030	0.029
Des El-Anz	14	12	13	0.26	0.24	0.30	0.023	0.030	0.027
Taify	11	12	12	0.27	0.28	0.28	0.021	0.023	0.022
Kamaly	14	12	13	0.15	0.29	0.22	0.020	0.020	0.020

Grape cultivars	Cu			Cd			Pb		
	2008	2009	Ave.	2008	2009	Ave.	2008	2009	Ave.
Soltany	13	11	12	0.26	0.27	0.27	0.022	0.032	0.027
White Shatta	14	12	13	0.19	0.25	0.22	0.021	0.033	0.027
Halawany	9	11	10	0.24	0.26	0.25	0.022	0.028	0.025
HSD 0.05	NS		5	0.10		0.06	NS		NS

The results representing fruit nitrate and nitrite content of the experimental cultivars are shown in Table 6. Fruit nitrate of Flame Seedless and Kamaly cultivars had similar and higher fruit nitrate content than all other cultivars, except Soltany and King Ruby. No significant differences were found among the Thompson Seedless, Red Glop, Perlette, Des El-Anz, Taify, White Shatta and Halawany cultivars in their fruit nitrate content. Additionally, the Kamaly, Flame Seedless and Soltany cultivars had similar and higher fruit nitrite content than King Ruby, Red Glop, Perlette and Thompson Seedless, which did not differ from each other in their nitrite content. Furthermore, Des El-Anz, Taify, White Shatta and Halawany had similar fruit nitrite content. A significant interaction effect between cultivars and years on fruit nitrite content was recorded for all studied cultivars.

Table 6

Fruit Ni, NO₃ and NO₂ content (mg kg⁻¹) of the different grape cultivars in 2008 and 2009 years

Grape cultivars	Ni			NO ₃			NO ₂		
	2008	2009	Ave.	2008	2009	Ave.	2008	2009	Ave.
Thompson Seedless	0.004	0.002	0.003	35	28	32	1.4	1.6	1.5
Flame Seedless	0.006	0.004	0.005	43	38	41	3.7	3.2	3.5
Red Glop	0.005	0.007	0.006	29	30	30	1.2	2.0	1.6
Perlette	0.004	0.003	0.004	27	42	30	1.5	1.6	1.6
King Ruby	0.008	0.005	0.007	38	33	36	1.4	1.8	1.6
Des El-Anz	0.004	0.005	0.005	31	27	29	2.0	2.2	2.1
Taify	0.004	0.004	0.004	26	32	29	3.0	2.0	2.5
Kamaly	0.003	0.005	0.004	47	38	43	3.5	3.8	3.7
Soltany	0.006	0.004	0.005	30	48	39	3.4	3.8	3.6
White Shatta	0.004	0.004	0.004	21	35	28	2.6	2.7	2.7
Halawany	0.004	0.003	0.004	29	37	33	1.9	2.5	2.2
HSD 0.05	NS		NS	10		7	NS		1.8

Discussion. The absorption of nutrients and water happen through the cultivar's root system and it rely on several factors such as the root structure (morphological and physiological features), the soil (availability of water and nutrients, temperature etc) and the above-ground parts of vines (ability of photosynthesis and transpiration of cultivars). These three factors interact with each other more or less and they are influenced by climate, techniques of cultivation and possible pathogens (Vercesi 1987). Similarly, Paranychianakis et al (2004) reported that mineral content of a grapevine is a combined result of the root system's ability to absorb, translocate and accumulate the different nutrients. Previous investigations had clearly stated the differences in nutrients uptake and content of many grape cultivars (Scienza et al 1986; Ruehl 1989; Brancadoro et al 1994; Bogoni et al 1995; Fardossi et al 1995; Kocsis et al 2001).

Furthermore, grape cultivars have shown differences in their nutrients uptake and distribution. These differences may be explained in different ways: First; cultivar may have different absorption capability or tendency for some specific minerals (Bavaresco et al 1991; Grant & Matthews 1996; Ruhl 2000). Second; differences in translocation and distribution of nutrients (Bavaresco & Lovisolo 2000). Third; hormone synthesis of cultivar roots and their translocation (Skene & Antdiff 1972). Fourth; some nutrients might be assimilated mostly by roots, thus reducing the amount translocated to the shoots. Keller et al (2001) discovered that over 85% of nitrogen was assimilated by way of vine root metabolism. Another important factor is the genetic origin of the

cultivar (Wolpert et al 2005; Russo et al 2010). Therefore, the differences in mineral content obtained in the present study among the different cultivars would be as a result of the mentioned factors.

In addition, some grape varieties may alter soil chemical characters and play a role in improving nutrients uptake. Brancadoro et al (1995) reported that the rootstocks of *V. labrusca* and scions grafted on them achieve a higher ability in uptaking iron, even in markedly alkaline soils. Such tolerant varieties can mobilize iron by reducing soil pH at root level, thanks to their ability to emit H⁺ and/or organic acids; in the latter case, iron is absorbed and transferred as a complex. Also roots of some cultivars can reduce Fe₃⁺ to Fe₂⁺ encouraging its migration from roots to leaves (Cinelli 1995).

In the meantime, little information is available that confirms or disputes the benefits of detrimental use of effluents for grapevines irrigation. Minerals usually enter the plant through root uptake and are concentrated most often in the root (Kabata-Pendias & Pendias 1992). In general, trace metal content decrease in the order of roots > stems > fruits > seeds (Kabata-Pendias & Wiacek 1985). Minerals uptake by plant is not only a function of the soil content, but it is also affected by the plants inherent affinity for a given mineral, which is highly controlled by type of fruit trees cultivars (Kabata-Pendias & Wiacek 1985). Accordingly, the data obtained confirm the various responses of the studied cultivars to the growing conditions and type of irrigation water at Riyadh region.

Conclusions. The results from this study suggested that significant differences existed in the leaf petioles elemental and some fruit pollutants concentrations among the grapevine cultivars analyzed that might be in due part to the geological status of the area under investigation, wastewater irrigation and the ability of cultivar to accumulate metals as well. This study could be also used as a reference for grape growers to help them decide the best varieties that might grow under Riyadh conditions giving the best growth and yield productivity.

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