MORPHOLOGICAL AND PHYSIOLOGICAL RESPONSES OF SOME OLIVE CULTIVARS SEEDLINGTO DIFFERENT CALCIUM CARBONATE LEVELS AND DROUGHT IN SOIL.

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ABSTRACT:

The Morphological and physiological responses of 6 olive cultivars seedlings namely; “Agezee”, “Koroneiki”, “Picual”, “Nebal”, “Manzanillo” and “Coratina” to three levels of calcium carbonate in soil (9.4%, 22% and 38.1% $CaCO_3$) and two irrigation levels which were 60% and 30% available water “A.W” were studied during two successive seasons, 2012 and 2013.The present study was achieved on one-year old seedlings grown in black plastic bags under saran green house conditions at the experimental farm of the Fac. of Agric. at Demo, Fayoum, Egypt.

A negative relationship was found between $CaCO_3$% in soil and/or deficit irrigation on growth Parameters of seedlings (plant height, leaf area, total shoot length/plant, dry weight/plant, root length density), total chlorophyll (mg/g fresh weight) and total carbohydrate %.

There were great differences among cultivars in their response to $CaCO_3$% in soil and drought. The cvs. that were most tolerant are “Nebal” followed by “Agezee” but “Coratina”, “Koroneiki” and “Manzanillo” were most susceptible; while “Picual” was intermediate tolerant.

INTRODUCTION:

Olive is one of the most important fruit crops cultivated in the Mediterranean area and of increasing interest world-wide. This species has long been cultivated in marginal lands (Connor and Fereres, 2005 and Therios, 2009).

Nowadays, fruit crop species are more and more influenced by various kinds of environmental stress. Drought and high lime levels of the soil are the most important kinds of abiotic stress (koyroetal.2012).

Calcareous soils occupy approximately 30% of the Earth's total land area. In Egypt, the calcareous soils constitute about 25 –30% of the total area according to Ministry of Agriculture estimation(2012).This area is one of the most promising areas for horizontal agriculture expansion in Egypt

Calcareous soil significantly reduced plant height of “Chemlali” olive although trunk circumference was increment than those grown in clay soil (Rokba, 1985).

Alcantara et al.(2003) evaluated “Arbequina”, “Cornezueio de jaen”, “Galega”, “Leccino”, “Lechindesevilla”, “Manzanilla de sevilla”, “Nevadillo negro” and “Pajarero” olive varieties grown in pots with high calcareous soil for their tolerance to lime induced chlorosis. They reported that there were differences in tolerance among olive cvs. and tolerance can be assessed by the relative chlorosis and growth parameters. Consequently, the varieties that were most susceptible were “Leccino”, “Arbequina”, “Lechin de sevilla” and “Galega” but “Nevadillo negro”, “Pajarero” and “Manzanilla de sevilla” cvs. was the most tolerant ; meanwhile “Cornezueio de jaen” cv. was intermediate tolerant.

Gruber and Kosegarten (2002) and Bavaresco et al.(2003) on grapevines grown in calcareous soil, found that plant growth is often considerably depressed, impaired formation of new leaves, characterized by a dramatic reduction in shoot growth and a distribution of dry matter towards to roots.

A high content of total lime in soil may result in the occurrence of grapevine chlorosis and show a negative effect on its growth, number of leaves, leaf area and weight of pruning wood. Also, the reduction of plant biomass of susceptible grapevine plants is related to reduce root growth due to high soil bicarbonate and to a lower photosynthesis rate which depend by a decrease of leaf chlorophyll (Diaz et al.2010 and Pavlousek, 2010).

Olive is a species showing a very good tolerance to drought, a severe stress may sometimes markedly influence qualitative properties and growth. When using plant material adapted to drought, it is possible to avoid losses by a severe water stress (Abd-El Samad 1995; and Van leeuwen et al. 2009).

Ben Ahmed et al.(2009) on olive mentioned that shoot elongation was varied consistently by following water application with values ranging between 33 mm and 353 mm for 20% Evapotranspiration (ETc), between 20 mm and 166 mm for 50% ETc and between 113 mm and 166 mm for 100% ETc.

Chehab et al.(2009)investigated the effect of different irrigation regimes on carbohydrate partitioning in leaves and wood of “Meski” and “Picholine” olive cultivars. Concentrations of carbohydrates in leaves and wood were strongly influenced by the irrigation regime. Ben Ahmed et al.(2008) showed that Significant differences were found in the soluble sugar content among cultivars in both leaves and roots according to water regimes.

The main goal of this research is to determine the effects of three levels of calcium carbonate in soil and two irrigation levels on growth of some olive cultivars seedlings to identify some cultivars or rootstocks that can bear high levels of calcium carbonate and drought.

MATERIALS AND METHODS:

This study was planned during two successive seasons (2012 and 2013) to study the effects of three levels of calcium carbonate (9.4%, 22% and 38.1%$CaCO_3$) and two irrigation levels which were 60% and 30% available water “A.W” on growth of one-year old seedlings of 6 olive cultivars namely; “Agezee”, “Koroneiki”, “Picual”, “Nebal”, “Manzanillo” and “Coratina”.

The present study was achieved under saran greenhouse conditions at the experimental farm of the Fac. of Agric. at Demo, Fayoum, Egypt. The soils of the growing media (9.4%, 22% and 38.1% $CaCO_3$) were taken from three different locations in the same experimental farm according to previous study on soil (Abdo, 2003).

Physical and chemical characteristics of the soil are shown in (tables, 1&2). The experiment included the following treatments:

- 9.4 % calcium carbonate ($CaCO_3$) + 60% available water A.W"(wet).
- 9.4 % $CaCO_3$+ 30% "A.W" (dry).
- 22 % $CaCO_3$+ 60% "A.W" (wet).
- 22 % $CaCO_3$+ 30% "A.W" (dry).
- 38.1 % $CaCO_3$+ 60% "A.W" (wet).
- 38.1 % $CaCO_3$+30% "A.W" (dry).

At last week of January of each season, 15 uniform and healthy one- year old seedlings of each cultivar were gained for each treatment, divided into 3 replicates (5 seedlings/replicate) and planted individually in black plastic bags (12 liter) filled with soils utilized as growing media. Al treatments were fertilized two times per season (in March and June) by nitrogen(Ammonium sulphate,20.6%N) using 50g./bag/season, Phosphorus (calcium triphosphate 15.5%P) and potassium (potassium sulphate, 48.5%K) were applied at the rates of 20 and 8g/bag/season, respectively.

The Experiment was designed as factorial experiments in complete randomized blocks design.

Vegetative growth parameters :

I: Morphological characteristics:

The following characteristics were measured at the end of October of each *season.*

1.1: Plant height(cm): The length from the soil surface to the highest growing point of plant.

1.2: Leaf area(cm^2):Thirty mature leaves were taken at random from the middle portion of the shoots/seedling; leaf area was estimated using digital plan- meter (model Planix, 7).

1.3: Number of shoots/plant: number of secondary branches/plant was counted.

Table(1). Physical properties of the studied soil samples:

sample	Physical characteristics								
	Particle size distribution			Texture	Ca co_3 %	Bulk density gm/cm^3	Soil moisture constants %		
	Sand %	Silt %	Clay%				F. C.	W.P.	A.W
1	69.7	15.5	14.8	Sandy loam	9.4	1.53	14.8	6.4	8.4
2	72.3	16.9	10.8		22	1.56	15.1	6.8	8.3
3	62.4	21.9	15.7		38.1	1.58	15.9	6.5	9.4

Table(2).Chemical properties of the studied soil samples:

sample	(Soluble cations (meq/L)				pH	ECe (ds/m)	Soluble anions (meq/L)			N %	K mg/kg	P mg/kg
	Ca^{++}	Mg^{++}	K^{+}	Na			Cl^{-}	HCO_3^{-}	SO_4^{--}			
1	105.2	0.80	1.11	38.0	7.28	3.6	104	8.0	33.0	0.14	410.7	35.2
2	59.92	0.84	1.02	41.0	8.06	4.12	40.0	8.0	122	0.056	165.1	2.30
3	75.73	1.36	0.98	46.0	8.13	4.5	44.0	4.0	75.5	0.028	174.6	1.60

1.4:Shoot length(cm):number of secondary branches/plant were counted and measuring for each seedling per replicate at the end of October from each season, then total shoot length per plant was calculated.

1.5: Dry weight of plant (g): one seedling was taken randomly from each replicate to determine weight of aerial portion (shoots and leaves) and roots, then dried in an electric oven at 70^{o}C to constant weight to estimate total dry weight of plant.

2. Chlorophyll(mg/g), representative leaf sample consisting of 30 leaves from spring cycle were taken in October for determination of total Chlorophyll. These pigments were Extracted by 80% acetone according to Arnon,1949.

3: Total carbohydrate percentage in leaves was determined according to the method described by Herbert et al.(1971).

4: Root length density(cm/cm3 of soil): at the end of each season, root length of one seedling/replicate of each treatment were carried out according to Newman, 1966.

5:Statistical analysis. Statistical analysis of the data was carried outaccording to Snedecor and Cochran (1994). Means were compared using LSD values of probability at 5% level.

RESULTS AND DISCUSSION:

and 4.95cm^2 in the Results tabulated in Table (3) revealed that plant height (cm) affected significantly by calcium carbonate % ($CaCO_3$) in soil, irrigation levels and cultivars. The highest value of plant height (84.72 cm) was obtained by seedlings grown at 9.4% calcium carbonate in soil followed in descending order by seedlings grown at 22% and 38.1% $CaCO_3$, respectively. Seedlings received the wet irrigation level (60%A.W.) gave the higher value of Plant height (89.93cm). Meanwhile, the lowest value in this respect(65.94 cm) was detected by seedlings exposed to dry treatment (30% A.W). This trend was true in both seasons.

Comparing the six cultivars under study, it is clear that “Coratina” and “Koroneiki” seedlings gave the highest significant values of plant height (96.78, 93.00 cm in the first season and 97.36, 93.33 cm in the second season), respectively. While, "Manzanillo" cv. show the shortest plant 60.39 cm and 57.78 cm in the two studied seasons, respectively. In this respect, (Rokba,1985) mentioned that the Plant height of olives grown in calcareous soil decreased comparing with those grown in clay soil.

Results clearly indicated that with decreased calcium carbonate levels in soil, leaf area increased significantly (Table,4).Seedlings grown at 9.4% $CaCO_3$ gave the biggest leaf area, but the smallest leaf area was achieved by seedlings grown at highest level of calcium carbonate. The differences between calcium carbonate levels in leaf area were significant in the first and second seasons. On the contrary, seedlings of wet treatment (60% A.W.) gave the highest values of leaf area (5.03cm^2 in the first season second season).

Teble 3

Teble 4

Meanwhile, the lowest values of leaf area were 4.36 cm^2and4.20cm^2 for seedlings grown under dry treatment in both seasons, respectively. Also, it is obviously clear from the data in(table,4) that seedlings of "Coratina" and "Koroneiki" cvs. grown at 9.4% $CaCO_3$ and 60% A.w. gave the highest significant values of leaf area in the two studied seasons. Meanwhile, seedlings of "Koroneiki" and "Picual" cvs. grown under dry treatment with the highest level of calcium carbonate produced the smallest leaf area in the first and second seasons. In this respect, Peston et al. (2003) on some fruit crops; Gruber and Kosegarten,(2002)on grapevines grown in calcareous soil, found that plant growth is often considerably depressed, impaired formation of new leaves and restricted leaf growth.

Data presented in (Table,5) show that the number of shoots/plant differed significantly by different $CaCO_3$ percentages, irrigation levels and cultivars. Consequently, seedlings grown at 9.4% $CaCO_3$ gave the highest number of shoots/plant followed in descending order by those grown at 22 and 38.1 $CaCO_3$%, respectively. Also, seedlings that received the higher value of irrigation level produced the highest number of shoots/plant (10.15 shoot in the first season and 9.91shoot in the second season).Meanwhile, number of shoot/plant for seedlings exposed to dry treatment decreased significantly by about 31.03% and 32.90% in the two studied seasons, respectively. Seedlings of "picual" cv. growing under wet treatment and 9.4 $CaCO_3$% had the highest number of shoots/plant. In additions, the lowest number of shoots/plant was detected by seedlings of "Agezee" cv. grown under higher levels of calcium carbonate and deficit water.

It is obvious from the data in Table (6) that the highest significant values of total shoot length/plant was 194.01 cm in the first season and 187.98 cm in the second season for seedlings grown at 9.4% calcium carbonate. Moreover, total shoot length/plant of seedlings grown at 22% and 38.1% $CaCO_3$decreased by 16.48 %, 26.38 % in the first season and by 11.14%, 26.58%in the second season, respectively. Total shoot length per plant decreased significantly by decreasing irrigation level, seedlings grown under dry treatments gave the lowest values of total shoot length per plant (137.74cm in the first season and 129.09cm in the second season) compared with those growing under wet treatments.

Values of the total shoot length of seedlings for "Agezee", "Koroneiki", "Picual", "Nebal", "Manzanillo" and "Coratina" grown at 9.4% $CaCO_3$% were 159.56, 211.93, 256.08, 165.48, 190.83 and 180.17cm, respectively. This values decreased by about 18.84, 28.04, 23.33, 14.90, 36.24 and 35.52 % when seedling grown at higher level of calcium carbonate in soil. As well as under dry treatments, the lowest decreasing rates of total shoot length (19.37 and 25.17%) of "Agezee" and "Nebal" seedlings, while the higher decreasing rate in this respect was 34.67% of "Koroneiki" seedlings. **This trend was** achieved in both seasons.

Teble 5

Teble 6

Peston et al. (2003) and Bavaresco et al. (2003) reported that the lime induced-chlorosis of some fruit crops and grapevines grown in high calcareous soil was characterized by a dramatic reduction of shoot growth. Also, Abd El Samed (2005) and Ben Ahmed et al. (2009) mentioned that annual shoot elongation decreased significantly by increasing the rate of water deficit.

Results presented in (Table,7) revealed that seedlings grown at 9.4% calcium carbonate produced the highest significant values of dry weight/plant (50.11g) in the first season and (52.37g)in the second season. This values decreased by about17.14% and 32.53% in the first season and by 15.14% and 30.99% in the second season for seedlings grown at22% and 38.1%$CaCO_3$, respectively. Also, dry weight affected significantly by irrigation levels, seedlings grown under dry treatment gave the least values of dry weight compared with those grown under wet treatment. Seedlings of “Picual” cv. had the highest significant values of dry weight per plant followed in descending order by seedlings of “Coratina”, “Koroneiki”, “Nebal”, “Manzanillo” and “Agezee” cvs., respectively. Significant differences were detected in the interactions between all the three studied factors (irrigation levels, $CaCO_3$% and cultivars).Also, the obtained results revealed that seedlings of “Nebal” cv. grown at higher level of calcium carbonate showed the lowest decreasing rate (21.56%) of dry weight/plant comparing with seedlings of “Koroneiki” and “Coratina” grown under the same conditions which were produced the highest decreasing rate of dry weight (42.27, 43.93%), respectively. This trend was true in the first and second seasons. Previous reports by Alcantara et al. (2003) reported that there are differences in tolerance among olive cvs. to high$CaCO_3$% in soil and tolerance can be assessed by the relative chlorosis and growth parameters. Abd - El Samad (1995) and Ben Ahmed et al. (2009) on olive cvs. mentioned that Vegetative growth of fruit trees is particularly sensitive to water deficit and there is a close relationship between moisture and organ enlargement.

Results in Table (8) revealed that total chlorophyll content influenced significantly by the levels of calcium carbonate, irrigation levels, cultivars. Also, a negative relationship was found between total chlorophyll in leaves and the percentage of calcium carbonate in soil. Consequently, leaves of seedlings grown at 38.1%$CaCO_3$had the lowest value of total chlorophyll (4.71mg/g fresh weight). This value increased by about by about 16.99% and by31.63% for seedlings grown at 22% and 9.4% $CaCO_3$, respectively. Moreover, leaves of seedlings grown under wet treatment contained the higher significant value of total chlorophyll compared with those grown under dry treatment. In addition, significant differences were detected in the interaction between irrigation levels and cultivars or between $CaCO_3$% and cultivars only in the first season. Leaves of “Picual” and "Nebal" cvs. grown under wet treatment with 9.4% $CaCO_3$ produced the highest values of total chlorophyll (8.01 and 7.62mg/g fresh weight), respectively. Meanwhile, the lowest values in this respect (3.23 mg/g fresh weight) were detected in leaves of "Agezee" cv., grown at 38.1% $CaCO_3$ and 30%A.W., respectively. This trend was true in both seasons. Previous reports of Abd El-Samad (1995) on olive, Abd El–Moteleb (1991)and Shawky et al.(1996) on grapevine concluded that total chlorophyll in leaves are increased by increasing irrigation level. Yoseph Levy, (1984)on citrus and Ksouri et al. (2005) and Pavlousek (2010)on grapevine mentioned that high concentration of bicarbonate in soil reduced leaf chlorophyll, peroxides enzyme activity and plant biomass.

Teble 7

Teble 8

Data tabulated in Table (9)showed that total carbohydrates in leaves affected significantly by the percentage of $CaCO_3$ in soil, irrigation level and cultivar. The higher percentage of total carbohydrates (31.65) was detected by seedlings grown at 9.4%$CaCO_3$, meanwhile leaves of seedling grown at soil contained the higher level of calcium carbonate had the lowest value of total carbohydrates percent. Also, leaves of seedlings exposed to dry treatment contained 27.21% of total carbohydrates, this percent increased by 15.91% for leaves of seedlings grown under wet treatment. Leaves of “Nebal” cv. showed the higher value of total carbohydrate and the lowest value in this respect was detected in leaves of "Coratina” cv. Regarding the interaction between the three studied factors, leaves of “Nebal” and “Agezee” seedlings grown under wet treatment with 9.4% $CaCO_3$contained the higher values of total carbohydrate. Meanwhile, leaves of “Agezee” seedlings grown under higher level of $CaCO_3$ and deficit irrigation had the least value in this respect. This trend was true in the two studied seasons. Chehab et al.(2009) noted that the concentrations of carbohydrates in leaves and wood of “Meski” and “Picholine” olive cvs. were strongly influenced by the irrigation regime. A high content of active and total lime in soil showed negative effect on its growth due to high soil bicarbonate and lower. photosynthesis rate(Abadía, 1992 and Pavlousek, 2010).

It is shown from the present results tabulated in Table (10) that root length density decreased significantly by increasing the rates of $CaCO_3$ in soil and irrigation deficit. Therefore, the highest significant value of root length density (0.97cm of roots/$cm^3$of soil) was achieved by seedlings grown at 9.4% $Caco_3$in soil, followed in descending order by those growing at 22% and 38.1%$CaCO_3$ in soil, respectively. Also, the value of root length density was 0.87 cm/cm^3 for seedlings grown under wet treatment, while it was 0.61cm/cm^3 of seedlings under dry treatment. Moreover, root length density differed by different studied cvs., seedlings of “Koroneiki” and “Coratina” cvs. produced the lowest values of root length density. Meanwhile, seedlings of “Nebal” and “picual” cvs. gave the highest values in this respect. This trend was true in the two studied seasons. Significant differences were detected between all treatments understudy. Consequently, seedlings of “Picual” and “Nebal” cvs. grown under wet treatment with 9.4% $CaCO_3$ had the highest values of root length density. On the other hand, the lowest significant values in this respect were achieved by seedlings of “Koroneiki” and “Picual” grown under dry treatment with higher values of calcium carbonate. This result is in harmony with Pavlousek, (2010) and Covarrubias and Rombola, (2013). They found that a high content of total lime in soil reduce root growth and decreased root biomass of grapevine.

From the previous results, It can be concluded that there are differences in tolerance among olive cvs. and the tolerance can be assessed by the relative chlorosis and growth parameters. Chlorosis are indicated by reduced leaf chlorophyll. Consequently, the cvs. that were most tolerant are “Nebal” followed by “Agezee” but “Coratina” , “Koroneiki” and “Manzanillo” were most susceptible, respectively. While “Picual” was intermediate tolerant.

Teble 9

Teble 10

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الإستجابات الفسيولوجية والمورفولوجية لشتلات بعض أصناف الزيتون لمستويات مختلفة من كربونات الكالسيوم والجفاف بالتربة.

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أجرى هذا البحث خلال موسمين متتاليين(٢٠١٢و٢٠١٣) لدراسة الإستجابات المورفولوجية والفسيولوجية لشتلات ستة أصناف من الزيتون هى عجيزى، كروناكى، بيكوال، نيبال، منزانيللوو كوراتينا. النامية تحت تأثيرثلاث مستويات مختلفة من كربونات الكالسيوم بالتربة(٩.٤%، ٢٢% و ٣٨.١% $Caco_3$) ومستويين من مستويات الرى (٦٠% ، ٣٠% من الماء الميسر).

وقد أظهرت النتائج:

وجود علاقة سالبة بين كل من نسبة كربونات الكالسيوم بالتربة ومعدل نقص مياه الرى من ناحية وبين قياسات النمو(ارتفاع النبات، مساحة الورقة، طول الأفرع الكلية لكل نبات ،الوزن الجاف، كثافة انتشار الجذور) وكذلك محتوى الأوراق من الكلورفيل والكربوهيدرات الكلية من ناحية أخرى.

وقد وجدت ايضاإختلافات كبيرة بين الأصناف فى مدى إستجابتها لكل من مستويات كربونات الكالسيوم بالتربة والجفاف حيث كان الصنف نيبال يليه العجيزى هم الأكثر تحملا بينما كانت أصناف الكورناكي والكوراتينا والمنزانيللو أكثر الأصناف حساسية على التوالى. وكان الصنف بيكوال متوسط التحمل .