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# Anatomical and biochemical characteristics of olive as influenced by *in-situ* moisture conservation during monsoon season

**N. Sharma* and Karan Singh**

Department of Fruit Science, Dr Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan 173 230, Himachal Pradesh

## ABSTRACT

**A field experiment for two years was undertaken to investigate the effects of different *in-situ* moisture conservation techniques, *i.e.*, $T_1$ - V-ditch (VD), $T_2$ - Cresent Bund with Open Catchment Pits (CBOC), $T_3$ - Trench System (TS) and $T_4$ - Traditional Basin System (BS) on anatomical and biochemical changes in olive cvs. Leccino and Cipressino grown under rainfed conditions. Significantly higher soil moisture levels were recorded at 30 and 60 cm depth in the basins of trees of both the cultivars maintained under CBOC and VD compared to those under BS. Stomatal conductance, stomatal size and stomatal density were also recorded significantly higher in trees under CBOC and VD. A significant increase in xylem vessel density and size of the individual vessel element were observed in the tree under CBOC, whereas, total soluble sugars, free amino acids and proline contents were significantly higher in the leaves in tree under BS. The treatment CBOC resulted in a higher increase in leaf malic acid, citric acid and free fatty acid contents over to those under BS. Overall, *in-situ* rain water conservation technique CBOC increased soil moisture levels and induced permanent histological changes in trees that may be useful for the adaptation of olive trees in the monsoon climate.**

**Key words:** Olive, *in situ* moisture conservation, anatomical, biochemical changes, monsoon season.

## INTRODUCTION

Olive (*Olea europaea* L.) is an evergreen tree native to the coastal areas of the eastern Mediterranean basin. It is of great economic importance in Mediterranean countries because of the oil extracted from its fruits (Orlandi *et al.*, 11). In India, olive cultivation is restricted to the states of Jammu and Kashmir, Himachal Pradesh, Uttrakhand and Rajasthan. In Himachal Pradesh, olives are grown on a limited scale in Kullu, Shimla, Solan and Sirmour districts. However, flower bud differentiation is of major concerns to the olive growers in the monsoon season because the yield is often irregular and uneconomical (Singh and Sharma, 17). The trees suffer acute moisture stress at their critical stages of growth and development during November-December and April through June due to erratic and uneven rains under rainfed conditions in the state. Water deficit situation restrict vegetative growth, hamper flower bud development and cause pistil abortion, which adversely affect olive production.

Conservation of rain water using *in-situ* moisture conservation techniques are absolutely necessary to enhance soil moisture content and improve proper flower bud development and fruit set. Soil working techniques- crescent bund with open catchment pits and V-ditch can be employed to conserve more soil moisture in rain fed conditions for improving growth and production of olives (Singh and Sharma, 17). The present study was undertaken to evaluate the influence of *in-situ* soil moisture conservation techniques on anatomical and biochemical status of olive tree to understand the mechanism of its adaptation in dry conditions.

## MATERIALS AND METHODS

Eighteen-year-old uniform trees of olive cvs. Leccino and Cipressino planted at 6 m × 6 m spacing in the Experimental Orchard of Department of Fruit Science, University of Horticulture and Forestry, Solan were selected for a two year study. Soil at the experimental site was silty loam having 6.02 pH, 0.697 $dSm^{-1}$ EC, 1.60% organic carbon content, 4.79% permanent wilting point, 21.06% field capacity (FC), and bulk density as 0.99 g $cm^{-3}$. Four soil working techniques used were; $T_1$ = V-ditch (Nagarin, 0.45 × 0.30 m), $T_2$ = Cresent bund (height: 0.3 m) with open catchments pits (3 pits of size 0.5 × 0.5 m), $T_3$ = Trench system (L × B × D- 3.0 m × 0.5 m × 0.5 m), $T_4$ = Traditional basins (2.0 m radius). All the treatments were replicated four times with one tree per replication in a randomized block design. Software SPAR-2 of IASRI, New Delhi was used for statistical analysis of data.

Soil moisture (%) were measured using AquaPro® soil moisture profiler at 15 day intervals from January 1 to July 1 at 30 and 60 cm depth of the access tubes

*Corresponding author's E-mail: nsharma.sol@gmail.com

fitted 1 m away from the trunk at two locations per tree basin and their mean values were computed (Singh and Sharma, 17).

Observations on stomatal conductance were recorded using IRGA (LiCOR 6200 photosynthetic meter) during morning (10:00-11:30 AM). Stomatal studies were carried out by preparing the leaf samples as per method suggested by Pathak *et al.* (12). For xylem vessel studies, shoot sections (1 cm) were taken from the current growth flush in the first week of July and fixed *in vacuo* with FAA (Formalin: acetic acid: alcohol) solution. The specimens were dehydrated in incremental series of ethanol and tertiary butyl alcohol (50% alcohol for 3 h, 70% alcohol for 3 h, 90% alcohol for overnight and 100% alcohol for 3 h) and embedded in paraffin. Samples were then sectioned by microtome at 15 µm and stained with safranine and fast green. Sections were examined under microscope. Total soluble sugars were estimated as per anthrone method (Wu and Xia, 20). Malic acid, citric acid, free fatty acids and free amino acids were estimated from the current flush growth in the first week of July as per standard methods (Thimmaiah, 18). Estimation of free proline was done ninhydrin reagent based assay (Bates *et al.*, 3). The statistical analysis of the two years pooled data was carried out as per method described by Gomez and Gomez (7).

## RESULTS AND DISCUSSION

During the study, significantly higher soil moisture was recorded under CBOP in comparison to the BS during both the years, irrespective of cultivars and soil depth (Table 1). It was noticed that leaf stomatal conductance, size and density were affected significantly by different soil moisture regimes (Table 2). Stomatal conductance was significantly higher in the trees under CBOC (2.19 mol $m^{-2}s^{-1}$) in comparison to trees under other systems of rain water harvesting. Trees under BS recorded the lowest leaf stomatal conductance (1.30 mol $m^{-2}s^{-1}$). Stomatal conductance has been reported to decline with increasing drought stress in olive (Dichio *et al.*, 5). Earlier, Tubeileh *et al*. (19) observed increased soil moisture content, leaf water content, leaf stomatal conductance and relative trunk growth

**Table 1.** Mean moisture contents as affected by *in-situ* moisture conservation techniques in olive.

| Treatment | Mean moisture (30 cm) 1st year | | | Mean moisture (30 cm) 2nd year | | | Mean moisture (60 cm) 2nd year | | |
|---|---|---|---|---|---|---|---|---|---|
| | Leccino | Cipressino | Mean | Leccino | Cipressino | Mean | Leccino | Cipressino | Mean |
| VD | 11.97 | 11.86 | 11.92 | 12.81 | 12.85 | 12.83 | 14.30 | 14.32 | 14.31 |
| CBOC | 13.05 | 12.96 | 13.01 | 13.74 | 13.86 | 13.80 | 15.12 | 15.38 | 15.25 |
| TS | 11.57 | 11.64 | 11.61 | 12.12 | 11.88 | 12.00 | 13.68 | 13.46 | 13.57 |
| BS | 11.06 | 10.71 | 10.89 | 11.27 | 11.23 | 11.25 | 12.11 | 11.99 | 12.05 |
| Mean | 11.91 | 11.79 | | 12.48 | 12.45 | | 14.04 | 13.80 | |
| $CD_{0.05}$ | T | 0.01 | | | 0.02 | | | 0.75 | |
| | V | 0.01 | | | 0.02 | | | 0.53 | |
| | T × V | 0.01 | | | 0.01 | | | 1.07 | |

**Table 2.** Leaf stomatal parameters as affected by different *in situ* moisture conservation techniques in olive.

| Treatment | Stomatal conductance (mol $m^{-2}S^{-1}$) | | | Stomata length (µm) | | | Stomata breadth (µm) | | | Stomatal density (No./microscopic field) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Leccino | Cipressino | Mean | Leccino | Cipressino | Mean | Leccino | Cipressino | Mean | Leccino | Cipressino | Mean |
| VD | 2.15 | 1.20 | 2.03 | 14.89 | 14.93 | 14.91 | 11.01 | 10.79 | 10.90 | 28.33 | 13.67 | 27.25 |
| CBOC | 2.33 | 1.92 | 2.19 | 15.04 | 14.99 | 15.01 | 11.10 | 10.95 | 11.03 | 28.50 | 27.50 | 28.09 |
| TS | 1.95 | 2.01 | 1.68 | 14.91 | 14.84 | 14.88 | 10.82 | 10.66 | 10.74 | 27.34 | 25.83 | 26.59 |
| BS | 1.29 | 1.37 | 1.30 | 14.82 | 14.79 | 14.80 | 10.65 | 10.74 | 10.69 | 27.17 | 24.83 | 26.09 |
| Mean | 1.93 | 1.33 | | 14.91 | 14.89 | | 10.89 | 10.79 | | 27.84 | 26.34 | |
| $CD_{0.05}$ | | | | | | | | | | | | |
| T | | 0.08 | | | 0.02 | | | | 0.01 | | | 1.21 |
| V | | 0.06 | | | 0.01 | | | | 0.01 | | | 0.85 |
| T × V | | 0.08 | | | 0.01 | | | | 0.01 | | | 1.20 |

rate in olive tree following water harvesting and summer irrigation. Increased shoot growth rates of olive trees under CBOC as reported earlier (Singh and Sharma, 17) could be related to higher stomatal conductance as noted in this study.

Leaf stomata size was largest (15.01 × 11.03 µm) under the treatment of CBOC and smallest (14.80 × 10.69 µm) in tree grown under BS (Table 2). A comparison among the cultivars revealed that Leccino had significantly higher stomata size than Cipressino. Drought stress has been reported to induce changes in leaf anatomy, such as decrease in stomata size, of olive trees (Bosabalidis and Kofidis, 4). In this study, trees under BS probably developed smaller stomata as a consequence of greater water stress (Table 1). Data clearly revealed significantly higher stomatal density in leaves from the trees with CBOC system (28.09 No./ microscopic field), than the tree maintained with BS (26.09 No./microscopic field), however in this respect, the CBOC treatment was not statistically different with that of VD (Table 2). Significantly, higher stomatal density was observed in cv. Leccino than the Cipressino. Higher stomatal density in the trees maintained under CBOC can be attributed to more soil moisture contents in comparison to other treatments (Table 1). Previous studies have reported greater stomatal densities on the dorsal surfaces of leaves from irrigated (100-600/ $mm^2$) in comparison to non-irrigated (20-30/$mm^2$) drought tolerant plants of olive, carob, pistachio, macadamia and *jojoba* (Baker and Procopiou, 2).

*In-situ* moisture conservation treatments also brought permanent histological changes in newly formed shoots of olives (Table 3). A significant increase in the average number of xylem vessels and diameter of individual vessel elements on cross section of shoots of trees under CBOC were observed when compared with the trees grown under BS. Trees under VD and TS were also superior to the trees under BS, in this regard. The decreased xylem vessel development under more water stress conditions (BS) in the present study corresponds well with the earlier results reported in pear (Sharma and Sharma, 14). Reduction in the number of xylem vessels and diameter of individual vessels offer more resistance to water movement through conductive tissues to the terminal parts of plants (Frakulli and Voyiatzis, 6). Significantly, more xylem vessel density and diameter of individual vessel element were observed in cv. Leccino compared to cv. Cipressino, which is in line with the earlier findings (Singh and Sharma, 14) that the former cultivar developed lesser negative leaf water potential in comparison to the later under different soil water regimes.

Total soluble sugars content of leaves was found significantly higher under BS system and lower under CBOC, conversely leaf malic and citric acid contents were noted significantly higher under CBOC system and lower under BS (Table 4). Cultivar Leccino had significantly higher total soluble sugars, malic acid and citric acid contents than Cipressino. When plants are subjected to water stress, they accumulate soluble sugars that are used for osmatic adjustment (Rhodes *et al.*, 13). Besides, sugars protect dried protein from denaturation as these serve as water substitute by satisfying the hydrogen-bonding requirement of polar groups of the surface of this dried molecule. It is possible then that under water stress conditions, activation of hydrolytic enzymes increased the rate of starch hydrolysis into soluble sugars, which would have caused depletion of starch and increase of total sugar content in leaves. Malic acid content of fruit has been correlated with soil moisture level, which was low in the fruits taken from the non-irrigated plot

**Table 3.** Anatomical characteristics as affected by *in situ* moisture conservation techniques in olive.

| Treatment | Xylem vessel density (No.) | | | Xylem vessel diameter (µm) | | |
|---|---|---|---|---|---|---|
| | Leccino | Cipressino | Mean | Leccino | Cipressino | Mean |
| VD | 153.00 | 149.67 | 151.3 | 49.67 | 47.67 | 48.67 |
| CBOC | 158.00 | 151.00 | 154.5 | 51.17 | 48.83 | 50.00 |
| TS | 151.67 | 147.67 | 149.7 | 48.33 | 47.67 | 48.00 |
| BS | 149.00 | 146.00 | 147.5 | 42.83 | 43.50 | 43.17 |
| Mean | 152.9 | 148.6 | | 48.00 | 46.92 | |
| $CD_{0.05}$ | | | | | | |
| | | T | 1.69 | | | 1.13 |
| | | V | 0.94 | | | 1.14 |
| | | T × V | 1.69 | | | 1.13 |

**Table 4.** Total soluble sugars, malic and citric acid contents as affected by *in situ* moisture conservation techniques in olive.

| Treatment | Total soluble sugars (%) | | | Malic acid (%) | | | Citric acid (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Leccino | Cipressino | Mean | Leccino | Cipressino | Mean | Leccino | Cipressino | Mean |
| VD | 3.18 | 1.95 | 2.56 | 0.21 | 0.20 | 0.20 | 0.22 | 0.19 | 0.20 |
| CBOC | 3.10 | 1.85 | 2.45 | 0.22 | 0.22 | 0.22 | 0.24 | 0.23 | 0.23 |
| TS | 3.17 | 1.94 | 2.53 | 0.22 | 0.18 | 0.20 | 0.23 | 0.18 | 0.20 |
| BS | 3.18 | 0.98 | 2.57 | 0.22 | 0.16 | 0.16 | 0.16 | 0.15 | 0.15 |
| Mean | 3.15 | 1.83 | | 0.21 | 0.16 | | 0.21 | 0.18 | |
| $CD_{0.05}$ | | | | | | | | | |
| | | T | 0.01 | | | 0.01 | | | 0.01 |
| | | V | 0.01 | | | 0.01 | | | 0.01 |
| | | T × V | 0.01 | | | 0.01 | | | 0.01 |

as compared to the fruits taken from irrigated plot (Kato *et al.*, 1985).

In this study, free fatty acid levels (Table 5) were higher in the trees under the treatments CBOC and VD systems, which maintained more soil water levels. However, free amino acids and proline contents (Table 5) were significantly more in leaves from the trees under BS (18.65 & 1420.5 µg/g, respectively) which recorded lower soil moisture level during the course of study, which are frequently observed responses to water stress (Simo-Sarkadi *et al.*, 16; Sharma and Sharma, 14). The minimum proline content (1256.5 µg/g) was found under the CBOC treatment, which was however, statistically at par with the treatment VD. Among the cultivars, leaves of Leecino accumulated significantly more amino acids and proline than Cipressino. Accumulation of organic compounds like amino acids notably proline in the cytoplasm make great contribution towards osmotic adjustment in water stressed plants. Besides, proline protects enzymes and cell membrane against adverse effects of oxidative stress (Agarwal and Pandey, 1). It accumulates to higher level than other amino acid in many plants under drought conditions (Morgan, 10). Higher accumulation of proline might help plants to tolerate dehydration by maintaining cell turgidity (Shivakumar *et al.*, 15) and protect plants against damages by reactive oxygen spices. Because of its capability to quench singlet oxygen and as a scavenger of $OH^-$ radicals, proline effectively protects protein, DNA and membrane from oxidative damage (Matysik *et al.*, 9).

The present study demonstrates that *in-situ* soil moisture conservation technique; CBOC improved soil moisture regime up to 60 cm of soil depth for olive trees, which can be a useful tool for adaptation of olives under rain fed conditions of monsoon climate. It was also observed that olive tree cvs. Leccino

**Table 5.** Leaf free fatty acid, free amino acid and proline contents as affected by *in situ* moisture conservation techniques in olive.

| Treatment | Free fatty acid (%) | | | Free amino acids (µg/g) | | | Proline (µg/g) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Leccino | Cipressino | Mean | Leccino | Cipressino | Mean | Leccino | Cipressino | Mean |
| VD | 0.62 | 0.56 | 0.59 | 17.73 | 18.74 | 17.80 | 1273.2 | 1242.9 | 1258.5 |
| CBOC | 0.62 | 0.57 | 0.60 | 17.27 | 17.68 | 17.32 | 1307.3 | 1277.3 | 1256.5 |
| TS | 0.60 | 0.55 | 0.58 | 17.56 | 9.50 | 18.01 | 1349.8 | 1370.9 | 1360.5 |
| BS | 0.58 | 0.53 | 0.56 | 18.50 | 17.96 | 18.65 | 1422.9 | 1417.8 | 1420.5 |
| Mean | 0.61 | 0.55 | | 17.74 | 17.58 | | 1338.5 | 1327.0 | |
| $CD_{0.05}$ | | | | | | | | | |
| | | T | 0.01 | | | 0.07 | | | 11.72 |
| | | V | 0.01 | | | 0.05 | | | 8.29 |
| | | T × V | 0.01 | | | 0.06 | | | 11.73 |

and Cipressino responded to changes in soil water regimes under different systems of *in-situ* moisture conservation through modification in development of xylem vessel elements and accumulation of osmolytes in their shoots and leaves. In future studies, these histological and biochemical markers can be useful for screening of water stress tolerant olive cultivars.

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