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Primary Metabolic Profile of Olive Leaves in Different Cultivars and Sites at Bikaner (Rajasthan)

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Abstract— *Due to the appreciable amounts of bioactive compounds in olive leaves and the effect of abiotic stress of arid area on their synthesis, this study evaluated the metabolic profile of primary metabolites in leaves of olive cultivars ('Arbequina', 'Barnea', 'Coratina' and 'Koroneiki') grown at two sites, viz., Lunkaransar and Napasar, located in Bikaner, Rajasthan (India). The total soluble carbohydrate content and protein content was determined by spectrophotometry. Lipid content was determined by solvent extraction and gravimetric method of quantification. Highest sugar content was found in 'Coratina' cultivar of Lunkaransar and protein content was found in 'Koroneiki' cultivar of Napasar. Maximum lipid content was found in 'Arbequina' cultivar of Napasar. Thus, it was possible to conclude that the cultivar and the site significantly affect the quantity of primary metabolites.*

Keywords— ***Abiotic stress, Cultivars, Mediterranean, Olive leaves, Primary metabolites.***

I. INTRODUCTION

Olive trees *(Olea europaea* L.) are commonly cultivated in the Eastern Mediterranean basin, particularly in coastal areas of South-eastern Europe, Northern Iran at the south end of the Caspian Sea, Western Asia, and Northern Africa. *Oleaceae* family includes 30 genera of deciduous trees and shrubs including olive trees. These are mostly grown in all continents except the Antarctica. The genus *Olea* belonging to *Oleaceae* family comprises 30 species but *Olea europaea* L. is the most popular member of the genus (Zohary et al., 2012). *Olea europaea* L. is an evergreen tree of 20-30 ft. in height with a spread of about 15-30 ft. with multiple stem. (Hashmi et al., 2015). *Olea europaea* L. has thousands of cultivars, with only a handful grown extensively

Olive development has taken place in Mediterranean regions but it is spreading in the rest of the world (Wang et al., 2018) (Porfirio et al., 2016). Olive trees are well known for their ability to adapt to arid to semi-arid regions due to its high tolerance and high adaptability to poor soils, drought salinity and excess of boron and chlorine (Bouwmeester et al., 2019). Olive tree leaves have been widely used in traditionally remedies, human diet as herbal tea and powder. They contain many primary potential bioactive compounds that may have antioxidant and anti-inflammatory properties (Haloui et al., 2010). In recent years, health effects of various herbal teas have been arising great interest. Olive-leaf is one of the most common, traditional herbal teas used among Mediterranean people to cure certain disease. Olive leaves are known for their potent antioxidant and anti-inflammatory properties and these properties are affected by primary metabolites whose level may be influenced by cultivar and place of production.

However, olive tree is native to Mediterranean climate but the climate of Rajasthan in North-western India resembles it. The phytochemical profile of plants can be influenced by the environmental conditions of the regions combined with intrinsic genetic factors (Ruiz Rodriguez et al., 2014; Maieves et al., 2015). According to the evaluation of the available literature our understanding of what affects quantity of primary metabolites in olive leaves still need more insight, together with the role of site where the tree is grown. For this reason, the aim of this research was to critically examine the influence of cultivar and site of cultivar on the quantitative property of primary metabolites of olive leaves by different methods. Diverse cultivars were compared for their primary metabolites at the same site to assess the role played by their genetic origin and also the

comparison was made between cultivars at two sites. In this study, primary metabolites extracted from olive leaves of four selected cultivars were analyzed at two different sites. Thus the present study emphasizes on better understanding about the primary metabolites found in response to prevailing environmental conditions in selected olive cultivars.

II. MATERIAL AND METHODS

The present study deals with the study of cultivars of *Olea europaea* L., viz., 'Arbequina', 'Barnea', 'Coratina' and 'Koroneiki' grown at Lunkaransar and Napasar sites of Bikaner in Rajasthan in which quantitative aspects of primary metabolites were studied. Fresh leaves of selected cultivars were collected, dried and pulverized for evaluation of different primary metabolites

2.1 Carbohydrate

2.1.1 Extraction of Carbohydrate

(a) Total soluble sugar

For extraction of total soluble sugar, 50 mg of each dried and powdered test samples was macerated with 20 ml of 80 % ethanol in mortar pestle. It was then left for 15 hours. The mixture was centrifuged for 12 min at 1500 rpm. The supernatants were collected and concentrated on a water bath. (Loomis and Shull, 1973). The volume of this concentration was made to 50 ml with distilled water (Extraction A).

(b) Starch

For extraction of starch, remnant pellets, after the centrifugation process of total soluble sugar, were collected and put in 5 ml of 52% perchloric acid and 6.5 ml of distilled water. It was shaken continuously for 5 min and centrifuged for 20 min at 2500 rpm. The process was replicated three times to collect the supernatant. (McCready et al. 1950). The volume of collected supernatant was made up to 100 ml (Extraction A).

2.1.2 Quantitative estimation of Carbohydrate

The total soluble sugar was quantitatively estimated using Phenol-Sulphuric acid method (Dubois et al.,1951). A stock solution of glucose (100 µg/ml) was prepared in distilled water and from this 0.1-0.8 ml was taken into eight test tubes and the total volume in each of the eight test tubes was adjusted to 1 ml by adding distilled water. 1 ml of each sample extraction A and extraction B were used to quantify the total soluble sugar. All sample test tubes and standard glucose concentration test tubes were left on ice chest. 1 ml of 5 percent phenolic solution was included in each test tube and shaken gently. Then 5 ml of concentrated Sulphuric acid was poured and tubes were shaken gently when acid was added. These solutions were then incubated in a water bath maintained at a temperature of 28°C for a duration of 20 minutes.

The optical densities (OD) of samples were taken at 490 nm by spectrophotometric analysis after setting for 100 percent transmission against the blank which is distilled water. A regression graph was prepared between known concentration of glucose and their respective absorbance by using law given by Lambert Beer. The concentration of total soluble sugar (mg/g dw) was calculated by using optical density (OD) of respective sample with standard glucose curve. Three replicates of each test sample were examined and their mean value were noted down and the results were expressed in mg/g dw of the sample.

2.2 Protein

2.2.1 Extraction of Protein

For the extraction of protein, 50 mg of all the eight dried and then powdered test sample were mixed in 10 ml of ten percent cold Trichloro acetic acid for 30 minutes and left at 4°C temperature for one day. All these solutions were centrifuged and upper parts were thrown. Remaining parts of all tubes were dissolved again in 10 ml of five percent Trichloro acetic acid and kept at 80°C on a water bath for 30 minutes. All these test samples were cooled down at room temperature, centrifuged again and the upper part of sample was thrown. This was done four times. The obtained residues were washed using distilled water and mixed in ten ml of 1N NaoH and kept at room temperature overnight (Osborne, 1962).

2.2.2 Quantitative Estimation of Protein

The total protein content was quantitatively determined by the assay given by Lowry et al. (1951). A stock solution of Bovine serum albumin was made in 1N NaoH (1 mg/ml). Eight concentrations ranged from 0.1 mg/ml - 0.8 mg/ml of the BSA solution were prepared in different test tubes by making the total volume of each tube to 1 ml after including distilled water. In another set of test tubes, 0.1 ml of all the eight samples were taken and made the solution up to 1 ml. To each test tube, two ml of freshly prepared alkaline solutions (made by adding 50 ml of 2 percent Sodium carbonate in 0.1 N NaoH and 1 ml of 0.5% $CuSO_4.5H_2O$ in Na-K tartrate) was included and left at room temperature for 10 min. Then in each test tube, 0.2 ml of Folin-Ciocalteau reagents was added and incubated at the room temperature (about 25ºC) for 30 minutes until the blue colour developed. Optical density (OD) were taken at 750 nm by spectrophotometer. Before taking the readings of the standard and the test samples spectrophotometer was set at 100% transmittance using blank. A standard curve was prepared with various concentrations of the standard Bovine serum solution against their respective absorbance,

following the Lambert-Beer's. The concentration of the total protein content in samples were calculated by using the optical density of each test samples with the standard graph. Three replicates of each test sample were examined and their mean value were noted down. The results were expressed in mg/g dw of the sample.

2.3 Lipids

2.3.1 Extraction of Lipid

For the Extraction and Quantification of lipid, 1 gm of each dried sample was crushed and mixed with 10 ml distilled water using a mortar and pestle (Jayaraman, J., 1981). The resulted paste was thoroughly mixed with a 30 ml of chloroform and methanol in the ration of 2: 1 in a conical flask. The mixtures were then left to stand overnight at room temperature. Subsequently, 20 ml of chloroform and an equal volume (20 ml) of distilled water were added, and the mixture was then centrifuged. The mixture was transferred into a separating funnel. The colored layer separated in methanol contained all the water soluble material. The lower chloroform layer was transparent and contained all the lipids. Three replicates of each test sample were examined and their mean value were noted down

2.3.2 Quantification of Lipid

The lower layer was taken in pre-weighed beakers. The chloroform layers dried in vacuum and weighed. Following evaporation, the weight of the beakers was re-measured. The weight difference was calculated and recorded as the total lipid content mg/g dw of the plant sample. This procedure was performed in triplicate, and the mean value was noted for each sample (Patel and Gaikwad, 2011).

III. RESULTS AND DISCUSSION

Highest sugar content 13.20 ± 0.09 mg/g dw was found in 'Coratina' cultivar of Lunkaransar and Second highest level of total sugar content 12.41 ± 0.07 mg/g dw was found in 'Coratina' cultivar of Napasar (Table 2) (Fig.1) Thus it can be estimated that 'Coratina' cultivar is comparatively better cultivar in view of sugar content in their leaves. Lowest total sugar content 9.74 ± 0.07 mg/g dw was found in 'Barnea' cultivar of Lunkaransar in the whole estimation. Second lowest sugar content 10.32 ± 0.072 mg/g dw was also found in 'Barnea' cultivar of Napasar. Thus 'Barnea' cultivar has overall minimum total sugar content. Similar results have also been obtained in previous studies where researchers found variability in sugar content in olive leaves from different cultivars (Medina et al., 2019; Romero *et al.,* 2017). Beligh et al. (2011) concluded that agronomic application of olive mill wastewater significantly increased the glucose and the fructose concentration when he studied the influence of irrigation with olive mill wastewater in the concentration of 30, 60, 100, 150 m^3h^{-1}.

Maximum protein content 27.48 ± 0.40 mg/g dw was found in 'Koroneiki' cultivar of Napasar. Second maximum protein 26.47 ± 0.40 mg/g dw was found in 'Koroneiki' cultivar of Lunkaransar. Thus 'Koroneiki' comparatively better cultivar for protein quantity in the whole estimation. Minimum protein content 21.42 ± 0.24 mg/g dw was found in 'Arbequina' cultivar of Lunkaransar in the whole estimation. Minimum protein content was not found in 'Arbequina' of Napasar (Table 2) (Fig.1). The variations may be due to difference of environmental conditions. Boudhrioua et al. (2009) analyzed the protein content of four varieties cultivated in Tunisia namely, 'Chemlali', 'Chemchali', 'Zarrazi' and 'Chetoui' and found protein content ranging from 1.05 to 1.30%. In another study, Erbay and Icier (2009) analyzed the protein content of olive leaves from the' Memecik' variety and found it to be 5.45%. Cavalheiro et al. (2015) studied the Protein content of olive leaves from five Brazilian varieties, namely, 'Ascolano', 'Arbosana', 'Negrinha do Freixo', 'Koroneiki', and 'Grappolo' and found that the protein content ranged from 10.50 to 13.10%. Diana et al. (2023) conducted qualitative and quantitative analysis on samples of 'Madural', 'Verdeal', 'Cobrancosa' leaves in different regions of Valpacos and Portugal and observed 3.45%, 4.01%, 2.15 % protein of fresh weight respectively. Thus there are variations in observed protein content in different cultivars at different sites.

Table 1: Sample names and Sample codes of Cultivars according to Study Sites

Sample name	Sample code	Study site	Cultivar
S1	NA	Napasar	'Arbequina'
S2	NB	Napasar	'Barnea'
S3	NC	Napasar	'Coratina'
S4	NK	Napasar	'Koroneiki'
S5	LA	Lunkaransar	'Arbequina'
S6	LB	Lunkaransar	'Barnea'
S7	LC	Lunkaransar	'Coratina'
S8	LK	Lunkaransar	'Koroneiki'

Maximum lipid content 66.14 ± 0.21 mg/g dw was found in 'Arbequina' cultivar of Napasar in the whole estimation. Second highest lipid content 65.66 ± 0.39 mg/g dw was found in 'Koroneiki' cultivar of Napasar. Minimum lipid content 61.15 ± 0.27 mg/g dw was found in 'Barnea' cultivar of Napasar (Table 2) (Fig.1). All these variations are due to different genetic makeup of cultivars. Infect, the same olive variety grown at different places shows a different fatty acid profile, as in, 'Arbequina' leaves from South of Rio Grande do Sul displayed higher linolenic acid (Cavalheiro et al., 2014) while 'Arbequina' leaves from Southern Minas Gerais contained higher Oleic acid. Thus, lipid profile in different cultivar at different sites has different profile.

Table 2: Primary metabolite content in leaves of selected olive cultivars

Sample name	Sample code	TSS (mg/g dw)	Proteins (mg/g dw)	Lipids (mg/g dw)
S1	NA	11.76 ± 0.15	25.33 ± 0.32	66.14 ± 0.21
S2	NB	10.32 ± 0.072	22.52 ± 0.20	61.15 ± 0.27
S3	NC	12.41 ± 0.07	23.29 ± 0.27	63.53 ± 0.40
S4	NK	11.30 ± 0.16	27.48 ± 0.40	65.66 ± 0.39
S5	LA	10.70 ± 0.18	21.42 ± 0.24	61.45 ± 0.40
S6	LB	9.74 ± 0.07	24.52 ± 0.35	63.55 ± 0.44
S7	LC	13.20 ± 0.09	22.58 ± 0.22	62.61 ± 0.45
S8	LK	10.44 ± 0.08	26.47 ± 0.40	65.47 ± 0.40

mg/gdw = milligram per gram dry weight; TSS = Total Soluble Sugars

Values are expressed as means ± SD of three samples; SD: Standard deviation

Sample names and Sample codes of Cultivars as per Table 1

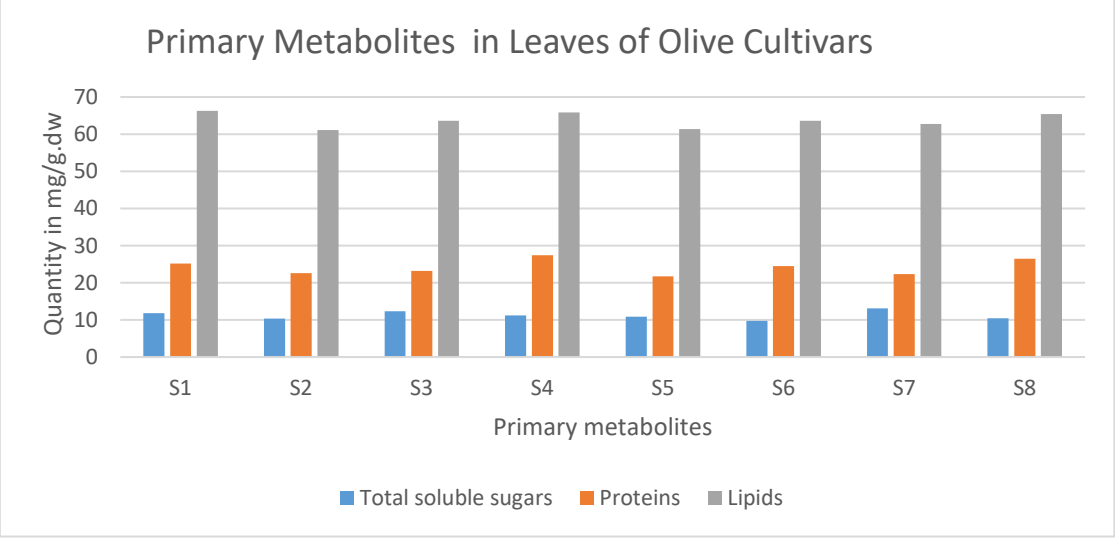

Fig.1: Primary Metabolites in Leaves of Olive Cultivars

mg/gdw = milligram per gram dry weight;

Sample names as per Table 1

IV. CONCLUSION

Primary metabolites from olive leaves are of interest to food industries, cosmetics and pharmaceutical. Each cultivar presented a different response to their site (micro-climate), in addition to its own metabolism. The values presented in this paper seem to indicate, in spite of limited number of number of cultivars analysed primary metabolites

composition might represent a useful contribution to biochemical characterization of olive leaves.

Moreover, the leaves of some cultivars could be interesting source of primary metabolites. High amounts of total soluble sugar have been found in 'Coratina' at both sites, whereas 'Koroneiki' is the richest source of protein at both sites. These results are applicable for the industries, as they are important information to enhance their processes. These findings could be used to manage production process and correlating the cultivar type and study site to the quantity of primary metabolite pattern.

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