Scientia Horticulturae 252 (2019) 138–148

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Guidelines for fertilizer use in pomegranate orchards based on seasonal uptake and partitioning of nutrients

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ARTICLE INFO

Keywords:
Punica granatum
Organ nutrient concentration
Organ nutrient amount
Nutrient removal

ABSTRACT

Understanding nutrient dynamics within the pomegranate plant on a temporal scale is critical to the development of sound nutrient management practices. This study investigated the seasonal patterns of nutrient uptake and redistribution in whole pomegranate plant in a 4-years old drip irrigated pomegranate cv. Bhagwa orchard of growing on sandy clay soil. In 2015-16, six plants were excavated each time during pre-pruning phase, flowering, fruit enlargement, fruit development and harvesting. Biomass, nutrient concentration and total nutrient amount of all plant organs were determined. Seasonal dynamics of total amount of N, P and S nutrients in plant share a consistent pattern: translocation of nutrients from woody organs to actively growing organs at the beginning of the season and nutrient movement to woody organs at the fruit maturity, while most of the canopy demand for K was met out from the soil uptake. Plant had higher total amount of Ca than all other nutrients throughout the growing season indicating its natural affinity towards Ca. Majority of Ca accumulated in permanent structures of the plant. The uptake pattern of macronutrients followed the order of Ca > N > K > Mg > S > P. Most of the uptake of N, K, Ca, Mg and S from the soil occurred between pre-pruning and bloom of the crop whereas the highest P uptake took place from fruit development to fruit maturity. The demand for micronutrients particularly Fe, Mn and B was highest during fruit enlargement stage while that of Zn was during the fruit development stage.

1. Introduction

Pomegranate (*Punica granatum* L.) is an economically important fruit crop of the tropical and subtropical region of the world which is valued for its delicious fruits rich in nutraceuticals (Badizadegan and Khabbazian, 1977). It has been of recent interest for its nutritional and antioxidant characteristics. Similar to other fruit crops, the yield and quality of pomegranate are influenced by the nutrient dynamics of the plant (Maity et al., 2017). The plant's mineral nutrient uptake from the soil each year is only a portion of the total plant's mineral nutrient annual need, the other portion is redistributed throughout the plant from woody and root tissues that function as storage organs (Pradubsuk and Davenport, 2010). An understanding of how the nutrient content of the plant varies throughout the season is central to define optimum condition for both crop yield and storage quality and in determining the timing and quantities of nutrients required by the plant. While only the seasonal changes in mineral nutrient composition in leaves and fruits are documented so far (Maity et al., 2017; Mirdehghan and Rahemi, 2007). Nutrient uptake and partitioning are strongly influenced by the plant's development stages (Lima et al., 2011; Nascimento et al., 2012). In most of the species, the macronutrients are usually considered as having high phloem mobility, except calcium (Ca) and sulfur (S). The nutrients uptake pattern and their redistribution are influenced by plant species also. The studies that investigate total nutrients amount in plant and their redistribution according to the plant developmental need are very much lacking in pomegranate, hindering the development of effective nutrient management schedule for optimizing plant nutrition and enhancing productivity. Extensive studies were carried out on this area in grapevine. Drawing reviews from those studies will provide an insight on nutrient dynamics that takes place with the perennial structures. Most studies report that grapevines take up the majority of the N between bloom and veraison (Bates et al., 2002; Hanson and Howell, 1995 and Mullins et al., 1992). It is also known that grapevines rely on stored nutrient reserves to supply early canopy development. Between 20–40 % of the annual N requirement of the canopy can be supplied from stored reserves in the trunk and roots with the greatest reliance on reserves occurring before bloom (Bates et al., 2002; Hanson and Howell, 1995; Williams, 1991). Less than 10% of annual vine

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https://doi.org/10.1016/j.scienta.2019.03.047
Received 29 June 2018; Received in revised form 2 February 2019; Accepted 23 March 2019

requirements for P, K, Ca or Mg have been reported to be remobilized from stored reserves in the trunk and roots of potted vines (Conradie, 1981). While the nutrient uptake capacity of vines can increase shortly after bud break, the majority of nutrient uptake from soil has most often been found to occur between bloom and veraison in both wine grapes (*Vitis vinifera* L.) and Concord grapes (*Vitis labrusca* L.) (Bates et al., 2002; Hanson and Howell, 1995; Lohnertz, 1991). Size and seasonal duration of vegetative, reproductive, and storage sinks differ with plant species and variable weather conditions (Robinson, 2005). Accordingly nutrient uptake pattern and their partitioning in different organs on temporal scale are likely to be different.

Therefore, the present study was undertaken to investigate the seasonal pattern of nutrient uptake and their redistribution in mature (4 years old) pomegranate plants.

2. Materials and methods

The study was conducted on mature (4-years old) pomegranate cv. Bhagwa orchard at ICAR-National Research Centre on Pomegranate research farm, Solapur, Maharashtra state, India, located at 17°48´ N latitude and 75°91´ E longitude, at an altitude of 457 m above mean sea level. Pomegranate air-layers were planted in 2012. The site was drip irrigated and managed with uniform fertilization, watering and pests management practices. The orchard soil is sandy clay isohyperthermic Lithic Ustorthent. Soil characteristics were: pH 8.1, free $CaCO_3$ 7.48%, organic carbon 1.87% and cation exchange capacity 20.50 cmol (p^+) kg^{-1} soil (Table 1).

During 2015-16, at pre-pruning, bloom (B), fruit enlargement (60 days after full bloom, DAFB), fruit development (120 DAFB) stages and harvest (180 DAFB), randomly six plants of uniform size were sampled each time (Table 2) using factorial experiment in completely randomized design. The above-ground plant was removed by cutting off the trunk at ground level and separated into trunk, branches, shoots, leaves, flowers/fruits (after bloom) plant organs. Roots under a single plant were extracted from the soil by gently digging the soil away from the root mass, maintaining connectivity of the roots and extracting the entire root ball. Moisture content of different organs was measured gravimetrically. Plant organs were washed thoroughly to remove extraneous materials, dried (at 65 °C, at least 48 h and until constant weight), weighed, ground and analyzed for nutrient elements.

Daily mean air and soil temperature and growing degree day (GDD) data were monitored by ICAR-National Research Centre on Pomegranate, Solapur, Maharashtra, India using data loggers (Model Opus-208 data logger, Lufft, Germany)

[y]Cumulative GDD between bloom and harvest

The samples were digested with H_2SO_4 to determine N and HNO_3/HCl in a microwave reaction system (mod. Multiwave 3000, Anton Paar, GmbH, Graz, Austria) to determine P, K, Ca, Mg, S, Fe, Mn, Zn and Cu. The samples were analyzed using spectrophotometer, flame photometer and atomic absorption spectrophotometer. The ground samples were dry ashed through combustion in microwave ashing system [mod. PYRO, Milestone Srl, Sorisole (BG), Italy] to determine B and were analyzed spectrophotometrically.

Table 1
Selected soil properties of studied pomegranate cv. Bhagwa orchard.

Soil properties	Values
Sand (%)	38.25
Silt (%)	12.61
Clay (%)	49.14
Organic C (g kg^{-1})	18.70
pH (soil:water, 1:2.5)	8.1
Free $CaCO_3$ (%)	7.48
Cation exchange capacity [cmol (p^+) kg^{-1}]	20.5
Available N (mg kg^{-1})	121.80
Available P (mg kg^{-1})	9.06
Available K (mg kg^{-1})	1240.00
DTPA extractable Fe (mg kg^{-1})	21.56
DTPA extractable Mn (mg kg^{-1})	40.38
DTPA extractable Zn (mg kg^{-1})	27.28
DTPA extractable Cu (mg kg^{-1})	12.34

An initial data analysis was conducted for two factors factorial experiment in completely randomized design using Proc GLM of SAS (version 9.2 for Windows; SAS Institute, Cary, NC, USA) to evaluate the influence of the main and interaction effects on organ nutrient concentration and total amount of nutrient (Table 3).

Additionally, total nutrient amount in plant was analyzed relative to nutrient concentration, plant dry weight and the interaction between concentration and dry weight. All were highly significantly correlated for N, P, K, Ca, Mg and S ($P < 0.01$) indicating a combination of plant mass and nutrient concentration both contributing to plant nutrient content. Data were subsequently analyzed using analysis of variance with SAS (version 9.2 for Windows; SAS Institute, Cary, NC, USA) to examine changes in dry weight and nutrient concentrations in all plant parts over time. Mean separation used least significant difference at $P < 0.05$. Nutrient uptake rate was computed by calculating the total nutrient amount of each macro-nutrient within each plant part (dry weight X concentration) divided by the number of days between sampling dates.

3. Results

3.1. Dry matter

Pomegranate plant above-ground biomass increased gradually from pre-pruning phase to flowering, followed by gentle increase up to fruit enlargement stage (0–60 DAFB), remained almost constant during 61–120 DAFB and then again increased sharply towards fruit maturity (Fig.1). The highest biomass recorded at harvest which is about 3.9 times of that at pre-pruning phase. The annual growth from shoots, leaves and fruits contributed significantly (58.02%) towards total above-ground biomass of plant at harvest. Maximum growth took place during fruit maturity period i.e. 121–180 DAFB followed by fruit enlargement period i.e. 0–60 DAFB. The least growth occurred during fruit development period i.e. 61–120 DAFB. While maximum fruit growth took place during fruit enlargement stage followed by fruit maturity stage. The growth of trunk occurred at two distinct phases, viz. initially at rapid rate up to 60 DAFB, then at reduced rate towards maturity of the fruits. The branches biomass remained almost stable up to 120 DAF and then it increased sharply during fruit maturity stage. Shoots and leaves biomass got diminished during fruit development stage, but they grew rapidly during maturity stage.

3.2. Seasonality of macro-nutrients concentration

Nitrogen concentrations in various plant parts decreased from bloom to harvest of fruits. Higher N concentrations were found in leaves and fruits, while lower N concentrations were noted in trunk and branches throughout the growing period (Fig. 2a). Fruit N concentration dropped rapidly during bloom to fruit enlargement stage (0–60 DAFB) and then remained almost stable during fruit development and fruit maturity stage. Woody organs viz. shoots, branches and trunk N concentrations initially decreased from pre-pruning phase to bloom thereafter it remained almost constant during late growth stage.

Phosphorus concentrations in woody organs viz. shoots, branches, trunk and roots were highest at pre-pruning phase and decreased to the lowest level at bloom (Fig. 3a). After that P concentrations in shoots and roots increased up to fruit development stage and remained constant thereafter, while in branches and trunk its concentration increased up to fruit enlargement stage, thereafter again declined towards fruit maturity stage. Like N, higher P concentrations were recorded in flowers/fruits and leaves while lower P concentrations were noted in

Table 2
Weather condition and sampling date according to phenological phases of pomegranate cv. Bhagwa in 2015-16 at experimental orchard, Solapur, India.

Growth stage	Sampling date	Growing degree day (> 10 °C)[y]	Mean air temperature (°C)	Mean soil temperature (°C)
Pre-pruning phase	6th October, 2015	–	16.60	33.50
Defoliation	17th November, 2015	–	15.10	31.54
Bloom	15th January, 2016	906	14.20	32.42
End of fruit enlargement	15th March, 2016	1944	18.10	36.41
End of fruit development	14th May, 2016	3344	24.70	36.30
Harvest	13th July, 2016	4541	16.40	29.05

Table 3
Significance [probability and least significant difference (LSD) values] of nutrient concentration and total amount of nutrients among different growth stages, organs as well as interactions between growth stage and organ of pomegranate cv. Bhagwa.

Parameter	Growth stage (GS)		Organ (O)		GS X O
	P	*LSD*	*P*	*LSD*	*P*
N concentration	< 0.0001	0.03	< 0.0001	0.03	< 0.0001
P concentration	< 0.0001	0.01	< 0.0001	0.01	< 0.0001
K concentration	< 0.0001	0.03	< 0.0001	0.03	< 0.0001
Ca concentration	< 0.0001	0.05	< 0.0001	0.05	< 0.0001
Mg concentration	< 0.0001	0.02	< 0.0001	0.02	< 0.0001
S concentration	< 0.0001	0.01	< 0.0001	0.02	< 0.0001
Fe concentration	< 0.0001	4.35	< 0.0001	4.77	< 0.0001
Mn concentration	< 0.0001	1.37	< 0.0001	1.50	< 0.0001
Zn concentration	< 0.0001	0.95	< 0.0001	1.04	< 0.0001
Cu concentration	< 0.0001	1.17	< 0.0001	1.28	< 0.0001
B concentration	< 0.0001	1.26	< 0.0001	1.38	< 0.0001
Total N amount	< 0.0001	0.46	< 0.0001	0.50	< 0.0001
Total P amount	< 0.0001	0.12	< 0.0001	0.13	< 0.0001
Total K amount	< 0.0001	0.38	< 0.0001	0.41	< 0.0001
Total Ca amount	< 0.0001	0.79	< 0.0001	0.87	< 0.0001
Total Mg amount	< 0.0001	0.29	< 0.0001	0.32	< 0.0001
Total S amount	< 0.0001	0.24	< 0.0001	0.26	< 0.0001

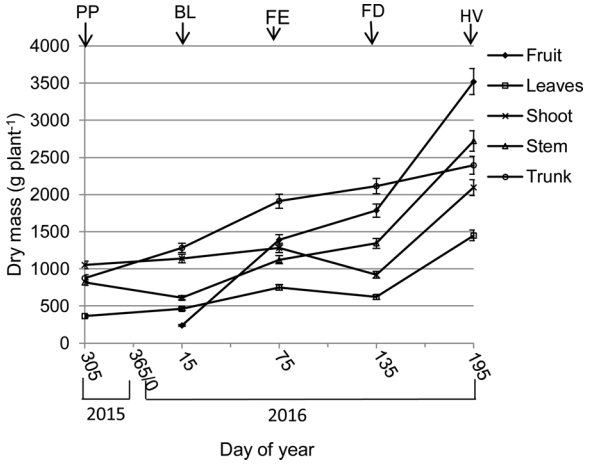

Fig. 1. Seasonal change in dry weight of various organs of 'Bhagwa' pomegranate. Arrows at the top of graph indicate the time of pre-pruning (PP), bloom (BL), fruit enlargement (FE), fruit development (FD) and harvest (HV). SE are represented as bars above and below the mean.

branches and trunk throughout the fruit growth period. Phosphorus concentration was highest in fruits at bloom and declined sharply during fruit enlargement stage then gradually during fruit maturity stage. Decreasing trend of P concentration was noticed in leaves with highest concentration observed during pre-pruning phase and lowest concentration at 120 DAFB i.e. end of fruit development stage. It was also observed that P concentrations in all organs except fruits got elevated during fruit enlargement stage (0–60 DAFB).

At bloom higher K concentration was found in fruits and lower K

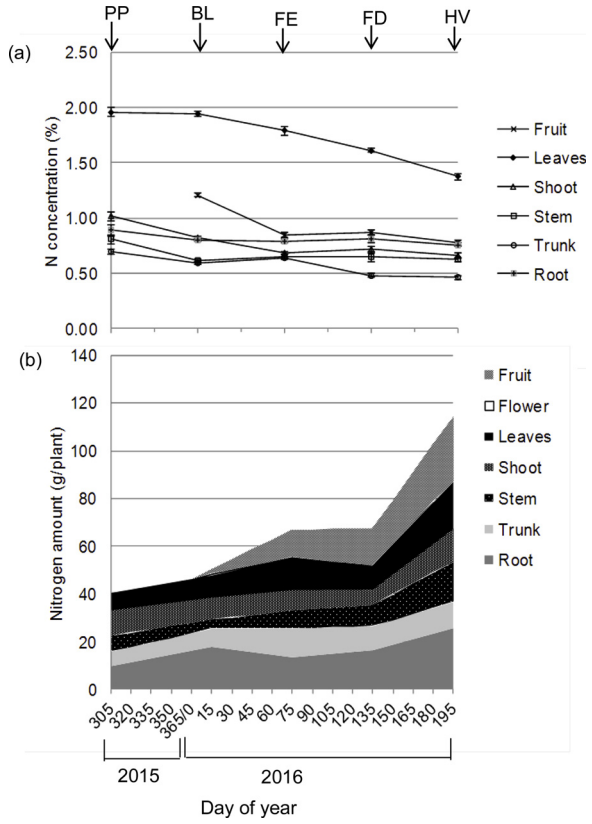

Fig. 2. Seasonal change in (a) concentration and (b) amount of nitrogen in various organs of 'Bhagwa' pomegranate. Arrows at the top of graph indicate the time of pre-pruning (PP), bloom (BL), fruit enlargement (FE), fruit development (FD) and harvest (HV). SE are represented as bars above and below the mean.

concentration was recorded in branches and trunk (Fig. 4a). Potassium concentration in fruits declined sharply during fruit enlargement stage followed by more gradual decrease during fruit development and fruit maturity stages, while leaves K concentration remained almost constant up to development stage and then increased during fruit maturity stage. In shoots, K concentration increased up to fruit development stage (120 DAFB) followed by sharp decline during fruit maturity. There was not much change in woody organs (branches, trunk and roots) K concentration with time of season.

Calcium was the most concentrated nutrient in branches and trunk at bloom (Fig. 5a). Its concentration in shoot and leaves significantly increased from bloom to fruit development stage, and then declined sharply during fruit maturity, while fruit Ca concentration declined gradually until the end of the season. Unlike N, P and K, higher Ca concentrations were recorded in woody organs (branches and trunk) and lower concentration was found in fruits.

Magnesium concentrations was higher and most dynamic in leaves, whereas its concentrations in other plant parts viz. shoots, branches, trunk and roots were much lower and showed little changes throughout

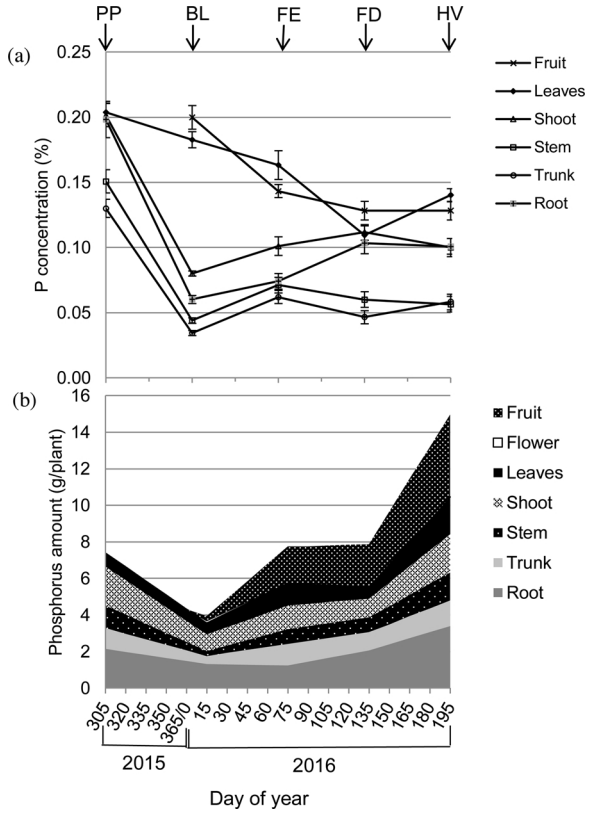

Fig. 3. Seasonal change in (a) concentration and (b) amount of phosphorus in various organs of 'Bhagwa' pomegranate. Arrows at the top of graph indicate the time of pre-pruning (PP), bloom (BL), fruit enlargement (FE), fruit development (FD) and harvest (HV). SE are represented as bars above and below the mean.

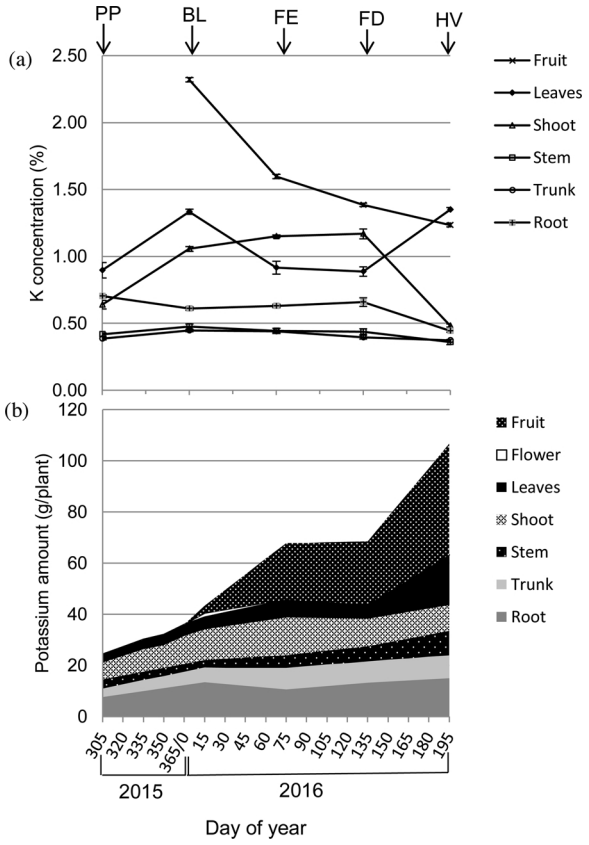

Fig. 4. Seasonal change in (a) concentration and (b) amount of potassium in various organs of 'Bhagwa' pomegranate. Arrows at the top of graph indicate the time of pre-pruning (PP), bloom (BL), fruit enlargement (FE), fruit development (FD) and harvest (HV). SE are represented as bars above and below the mean.

the growing season (Fig. 6a). Like Ca, magnesium concentration in leaves increased up to fruit development stage and then declined sharply towards fruit maturity. While its concentration in fruits increased during initial growth period (i.e. up to 60 DAFB) and then remained almost constant during fruit development and maturity stages. However, in shoot Mg concentration increased up to bloom and then continued to decline throughout the fruit growth period.

Sulphur concentrations in shoot and leaves were much higher than in fruits (Fig. 7a). Its concentration increased from pre-pruning phase to bloom and then declined during fruit enlargement and fruit development period and again increased in shoot while sharply decreased to very low level in leaves during fruit maturity. Fruit S concentration initially dropped rapidly followed by more gradual decrease during fruit maturity. Sulphur concentrations in branches and roots increased during pre-pruning phase and then declined gradually after bloom, while its concentration in trunk showed somewhat rising trend throughout the growth period.

3.3. Macro-nutrient uptake and partitioning

Total amount of nitrogen in pomegranate plant increased slowly from pre-pruning phase to bloom and then at moderate rate during fruit enlargement stage, remained constant during fruit development stage and then again increased sharply during fruit maturity stage (Fig. 2b). Highest amount of N uptake (43.61% of the total) occurred during pre-pruning to bloom period followed by that took place during fruit maturity (41.28% of the total) and then during fruit enlargement stages (14.84% of the total) (Table 4). Nitrogen uptake rate was high during fruit maturity stage than during fruit enlargement stage (Table 5).

Very meager amount of N was taken up during fruit development stage. During pre-pruning to bloom period the amount of N in woody organs viz. trunk, branches and shoots changed little with the exception of cognizable increase of N amount in fruits, leaves and branches during fruit enlargement stage, while its amount decreased in roots and shoots during the same period. The amount of nitrogen continued to increase in fruit and began to increase in roots while its amount decreased in leaves, shoots and trunk during fruit development stage. During fruit maturity stage, N content increased in all the plant parts except in trunk, however the extent of increase was much higher in shoots and leaves.

At harvest highest amount of N was recorded in fruits (20.15 kg N ha^{-1}) followed by roots (18.87 kg N ha^{-1}) (Table 6). The total amount of N found in different parts of fully grown pomegranate plant (4-year old) was 84.58 kg ha^{-1}. Approximately, half of N in plant was found to confine in fruits, leaves and shoots (45.12 kg ha^{-1}).

Unlike N, total phosphorus amount decreased from pre-pruning phase to bloom and then increased during fruit enlargement stage, remained constant during fruit development stage and again increased sharply during fruit maturity stage (Fig. 3b). Majority uptake of P took place during fruit maturity stage (47.18% of the total) followed by that occurred during pre-pruning to bloom period (26.46% of the total) and fruit enlargement stage (25.67% of the total) (Table 4).

Phosphorus uptake rate during fruit maturity stage was almost 1.4 times of that recorded during fruit enlargement stage (Table 5). The amount of phosphorus declined in shoots, branches, trunk and roots during pre-pruning to bloom period, while its amount in leaves remained constant. After bloom, the amount of P increased in fruits, leaves, shoots and trunk while it continued to decrease in roots during fruit enlargement stage. Very little changes in amount of P were noticed

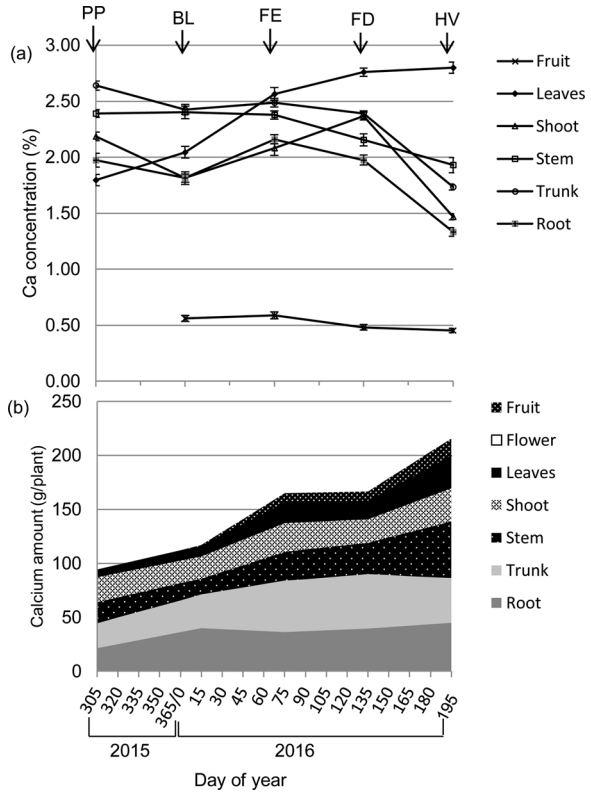

Fig. 5. Seasonal change in (a) concentration and (b) amount of calcium in various organs of 'Bhagwa' pomegranate. Arrows at the top of graph indicate the time of pre-pruning (PP), bloom (BL), fruit enlargement (FE), fruit development (FD) and harvest (HV). SE are represented as bars above and below the mean.

in leaves (decreased) and roots (increased) during fruit development stage. During maturity stage the amount of P increased in all parts except in trunk. At harvest, highest amount of P was recorded in fruits (3.33 kg P ha^{-1}) followed by in roots (2.52 kg P ha^{-1}), while total amount of P in fully grown plant was found to be 11.07 kg P ha^{-1}(Table 6).

Total amount of potassium in plant followed similar pattern as that observed with N throughout the growing season (Fig. 4b). Here, also highest amount of K (40.61% of the total) was taken up during pre-pruning to bloom period and it was followed by that took place during fruit maturity (35.78% of the total) and fruit enlargement (23.60% of the total) stages (Table 4). Uptake rate of K was found to be higher during fruit maturity stage than fruit enlargement stage (Table 5). Negligible uptake of K took place during fruit development stage. The amount of K in roots, trunk and shoots increased while it decreased in branches and remained constant in leaves during pre-pruning to bloom period. After bloom, the amount of K increased in fruits, leaves and shoots while it decreased in roots up to fruit enlargement stage. Then amount of K in different plant parts remained almost constant up to 120 DAFB and then again increased during fruit maturity stage. Enhancement of K amount in fruits and leaves were predominant during this stage. At harvest highest amount of K was confound in fruits followed by in leaves (Table 6). The total amount of K in full grown plant was 78.97 kg ha^{-1}which closely followed the amount of N in plant.

Like N and K, total amount of calcium in plant increased slowly during pre-pruning phase to bloom and then rapidly during fruit enlargement stage, remained stable during fruit development stage and then again increased slowly during fruit maturity stage (Fig. 5b). Majority Ca uptake occurred during pre-pruning to bloom period (61.10% of the total) and rest of the uptake took place during fruit enlargement (24.95% of the total) and fruit maturity stages (13.04% of the total) (Table 4). Highest Ca uptake rate was recorded during fruit enlargement stage which was followed by that during fruit maturity stage (Table 5). The amount of calcium increased in leaves, trunk and more prominently in roots and decreased in stem during pre-pruning phase to bloom. After bloom it increased in all plant parts except roots where it remained constant during fruit enlargement stage. The amounts of Ca were much higher in woody organs, particularly in trunk and branches than in leaves and fruits. It again increased in stem, shoots and fruits and decreased in leaves and trunks during fruit maturity stage. Unlike, N, P and K, higher amount of Ca were recorded in branches (38.82 kg ha^{-1}), roots (33.21 kg ha^{-1}) and trunk (30.69 kg ha^{-1}) than fruits, the eatable part (11.78 kg ha^{-1}) at harvest (Table 6). The total amount of Ca recorded in full grown plant was 141.91 kg ha^{-1} which was much higher than the amount of primary nutrients like N and K recorded in this study.

Total amount of magnesium in plant increased from pre-pruning phase to the end of fruit enlargement stage (60 DAFB), remained constant during fruit development stage and then again increased during fruit maturity stage (Fig. 6b). More than half of the Mg uptake occurred during pre-pruning phase to bloom period and rest of the uptake took place during fruit maturity (28.57% of the total) and fruit enlargement stage (18.62% of the total) (Table 4). Among the three fruit growth stages, Mg uptake rate was highest during fruit maturity stage followed by that during fruit enlargement stage (Table 5). During pre-pruning phase to bloom period, the amount of Mg increased in leaves, shoots and roots and decreased in branches. After bloom, it increased in fruits, leaves, branches and trunk while decreased in roots during fruit enlargement stage. Subsequently, its amount increased in roots and fruits, while decreased in trunk, shoot and leave and thus maintained a stable Mg amount in plant during fruit development stage. Further, the amount of Mg increased in all plant parts, more prominently in fruits except in leaves and trunk during fruit maturity stage. Significantly higher Mg amounts were recorded in fruits (7.92 kg ha^{-1}) and roots (7.97 kg ha^{-1}) at harvest sharing more than half of the total amount of Mg in plant. Total amount of Mg in a full grown plant was 30.15 kg ha^{-1} at harvest (Table 6).

Unlike other macro-elements, total amount of S in plant increased from pre-pruning phase to bloom, remained almost constant during fruit enlargement and fruit development stages and again increased during fruit maturity stage (Fig. 7b). Majority uptake of S occurred during pre-pruning to bloom period (54.93% of the total) and during fruit maturity stage (42.54% of the total) (Table 4). Sulphur uptake rate was found to be highest during fruit maturity stage (Table 5). The amount of S increased in all plant parts during pre-pruning phase to bloom. After bloom it continued to increase in leaves, branches, trunk and fruits but decreased in roots and shoots during fruit enlargement stage. However, during fruit development stage the amount of S increased in trunk and decreased in shoots while remained almost constant in other plant parts. Subsequently, its amount again increased in all plant parts except in leaves during fruit maturity stage. Unlike other macro-elements, majority amount of S was confound in shoots (5.75 kg ha^{-1}, equivalent to 24% of the total) and roots (5.75 kg ha^{-1}, equivalent to 24% of the total), while only 9.34% of total S was found in fruits at harvest (Table 6). Total amount of S in a fully grown plant at harvest was 23.96 kg ha^{-1}.

3.4. Seasonality of micronutrient concentration

The concentration of micronutrients (Fe, Mn, Zn, Cu and B) within various plant parts changed significantly over time. Iron concentration in fruit decreased during fruit enlargement stage and then gradually increased during rest of the fruit growth period (Fig. 8a). While in leaves it increased up to fruit enlargement stage and then continued to decrease during fruit development and maturity stage. Iron concentrations in stem and trunk increased during rest period to bloom and then declined throughout the fruit growth period, while in roots it

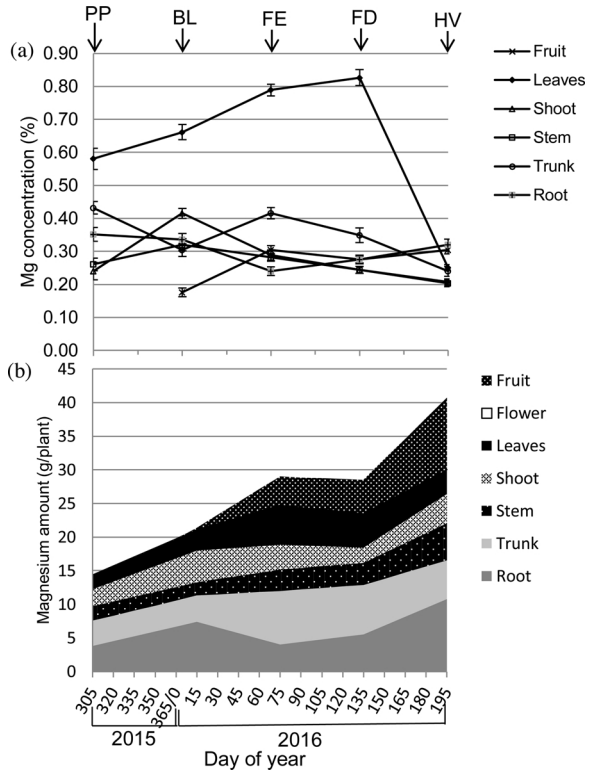

Fig. 6. Seasonal change in (a) concentration and (b) amount of magnesium in various organs of 'Bhagwa' pomegranate. Arrows at the top of graph indicate the time of pre-pruning (PP), bloom (BL), fruit enlargement (FE), fruit development (FD) and harvest (HV). SE are represented as bars above and below the mean.

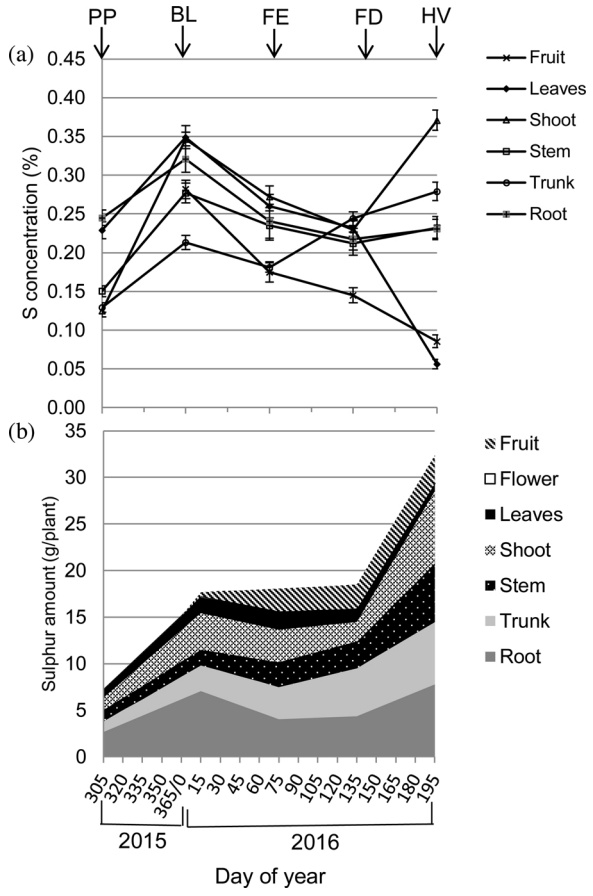

Fig. 7. Seasonal change in (a) concentration and (b) amount of sulphur in various organs of 'Bhagwa' pomegranate. Arrows at the top of graph indicate the time of pre-pruning (PP), bloom (BL), fruit enlargement (FE), fruit development (FD) and harvest (HV). SE are represented as bars above and below the mean.

initially decreased and then increased during fruit enlargement stage followed by declining during rest of the fruit growth period. Highest Fe concentrations were recorded in roots while lowest Fe concentrations were noted in fruits throughout the growth period. Manganese concentration in fruits, leaves, shoots, stem and trunk followed similar trend as that of Fe (Fig. 8b). In contrast, Mn concentration in roots continued to increase from rest period to fruit development stage and then declined gradually during fruit maturity stage. Highest Mn concentrations were recorded in leaves while lowest concentrations were noted in fruits throughout the growth period. Unlike Fe and Mn, zinc concentration in fruits and leaves increased during fruit enlargement stage and then declined during fruit development stage followed by slight elevation in fruit and reduction in leaves during fruit maturity stage. Zinc concentrations in woody tissues viz. shoots, stem, trunk and roots also followed similar trend as that recorded in leaves (Fig. 8c). Highest Zn concentrations were found in shoots while lowest concentrations were recorded in fruits during the growth period. Copper concentration in fruit remained almost constant during fruit growth period while in other parts, it increased during rest period to bloom and then declined during fruit growth period (Fig. 8d). Like Mn, highest Cu concentrations were noted in leaves while lowest concentrations in fruits.⇒ Unlike other micronutrients, boron concentrations in various plant parts decreased from bloom to fruit development stage and then increased slightly in all parts except

χSeasonal change in concentration of (a) iron (b) manganese (c) zinc and (d) copper in various organs of 'Bhagwa' pomegranate. Demarcations at the top of graph indicate the time of pre-pruning (PP), bloom (BL), fruit enlargement (FE), fruit development (FD) and harvest (HV). SE are represented as bars above and below the mean.

leaves where it continued to decrease during fruit maturity stage (Fig. 9). However, changes in the concentration of B in various plant parts did not show any clear differentiation. Fully grown plants were found to have highest content of Fe (3348.32 g ha^{-1}) followed by Cu (322.45 g ha^{-1}), B (255.75 g ha^{-1}), Mn (198.76 g ha^{-1}) and Zn (149.42 g ha^{-1}) at harvest (Table 7).

4. Discussion

4.1. Macronutrients

In pomegranate it has been usual to find leaves nutrient concentrations outside the published standard ranges even in orchards with apparent normal growth. However, leaves nutrient concentrations at flowering reported in this study were not greatly dissimilar to those found in the literature (Gosavi et al., 2017; Raghupathi and Bhargava, 1998) which suggested that the nutritional status of the plants used in this study is within the adequate range for the nutrients considered.

Leaves, shoots, branches and fruits had higher N concentrations early in the season and then these declined throughout the fruit growth period. These trends are consistent with results reported from numerous studies in grapevine (Schreiner and Scagel, 2006; Pradubsuk and Davenport, 2010; Boselli et al., 1998). As far as we know, there are no published data analyzing the various parts of pomegranate plant for nutrient concentration and their amount at temporal scale. Nitrogen concentrations in leaves and shoots usually decreases as growing season progress (Peuke, 2009) likely due to dilution effect or remobilization to fruits and seeds. After harvest plant growth is reduced or nil and remobilization to fruits ends. The amount of N in roots was high at bloom, declined until fruit enlargement (up to 60 DAFB) and then increased up

Table 4
Seasonal uptake of nutrients calculated from the change in total amount each nutrient in pomegranate plant between growth stages of Ambia bahar crop (January-February flowering).

Nutrient	Avg. total uptake (g plant^{-1})	Plant uptake (% of total)				Significance by growth stage (*P*)
		Up to flowering	Flowering to fruit enlargement (0-60 DAF)	Fruit enlargement to development (61-120 DAF)	Fruit development to maturity (121-180 DAF)	
N	114.30	43.61^{a*}	14.84^{c}	0.27^{d}	41.28^{b}	< 0.0001
P	14.96	26.46^{b}	25.67^{b}	0.70^{c}	47.18^{a}	< 0.0001
K	106.70	40.61^{a}	23.06^{c}	0.55^{d}	35.78^{b}	< 0.0001
Ca	226.05	61.10^{a}	24.95^{b}	0.91^{d}	13.04^{c}	< 0.0001
Mg	40.74	52.81^{a}	18.62^{c}	0^{d}	28.57^{b}	< 0.0001
S	32.37	54.93^{a}	1.14^{c}	1.39^{c}	42.54^{b}	< 0.0001

* Results within a column are significantly different (LSD at $P < 0.05$) when letters following the value differ.

Table 5
Uptake rate of nutrients during different fruit growth stages by pomegranate plant during Ambia bahar crop (January-February flowering).

Nutrient	Uptake rate (mg plant^{-1}day^{-1})			Significance by growth stage (*P*)
	Flowering to fruit enlargement (0-60 DAF)	Fruit enlargement to development (61-120 DAF)	Fruit development to maturity (121-180 DAF)	
N	376.46^{b*}	5.23^{c}	786.84^{a}	< 0.0001
P	84.79^{b}	1.73^{c}	118.27^{a}	< 0.0001
K	546.29^{b}	9.72^{c}	637.14^{a}	< 0.0001
Ca	1060.84^{a}	29.14^{c}	421.39^{b}	< 0.0001
Mg	169.64^{b}	0^{c}	204.43^{a}	< 0.0001
S	8.27^{b}	7.53^{b}	231.38^{a}	< 0.0001

* Results within a column are significantly different (LSD at $P < 0.05$) when letters following the value differ.

to harvest. This data shows that substantial recharging of pomegranate N reserve in roots occurs after harvest and may continue even after leaves are shed. This is in consonance with that observed in grapevine by Bates et al. (2002) who found that N concentration in fine roots increased from bud-break to bloom.

Changes in the amount of N within the canopy of pomegranate showed that leaves were the most important sink after fruits for N accumulation as observed in grapevine (Schreiner and Scagel, 2006; Williams, 1987). Nitrogen uptake from soil and remobilization of stored N were needed to supply the developing canopy of pomegranate. The largest fraction of stored N used to support canopy development came from large woody roots and trunks. In grapes, other have found that N was primarily used from the trunk and large woody roots (Lohnertz, 1991; Schreiner and Scagel, 2006; Pradubsuk and Davenport, 2010). We also found that remobilization of N from the roots and trunk to the canopy was more pronounced after bloom than before bloom which is in agreement with previous reports in grapevine (Schreiner and Scagel, 2006).

The fruit contained 20.15 kg N ha^{-1} representing 1.95 kg N per tonne of fresh fruit. Though, on average N removed per tonne of fruit was of higher magnitude to the values reported in other variety of pomegranate, 'Ganesh' by Raghupathi and Bhargava (1996), which may be because of varietal characteristics, this is low values (χ25–27% of other fruit crops' removal) if compared to amounts of N usually removed by other fruit crops such as mango (6.7 kg/ton), banana (5.6 kg/ton), citrus (9.0 kg/ton) and grapes (8.0 kg/ton) grown in this semi-arid eco-region (Ganeshamurthy et al., 2011). The nutrients removed in the fruit are an integral loss to the soil-plant system. About 22% of N (14.74 kg ha^{-1}) in the above ground part of the plant was in the leaves at harvest time. Approximately 50% of this N disappeared during senescence which may mean that it was remobilized to the perennial structures and or lost through volatilization from the canopy (White, 2012; Eichert and Fernandez, 2012). Nitrogen still present in the fallen leaves can undergo mineralization in the soil and be taken up by the roots or lost through NH_3 volatilization or denitrification. Nitrogen present in shoots (10.23 kg ha^{-1}) can follow the same route if prunings are left on the ground. If prunings are removed their N is lost from the soil-plant system. The ground management of the orchard may also be of great importance. Cover crops, for instance, can compete with the pomegranate for inorganic N in the soil (Celette et al., 2009; Celette and Gary, 2013) curtailing the efficiency of N recycling. Based on N uptake and redistribution pattern, it is implicit that fertilizer supplement should be made in 3.0:1.0:2.7 ratio during pre-pruning phase, fruit enlargement stage (0–60 DAFB) and fruit maturity stage (121–180 DAFB). Fertilization is not required during fruit development stage (61–120 DAFB) as uptake is negligible during this period.

Fruits and leaves of pomegranate plant had higher concentration of P throughout the fruit growth period. This implies that phosphorus is mobile element within the pomegranate plant which can readily get translocated (Mullins et al., 2007; White, 2012). In leaves and fruits P decreased over the growing season and similar trend was reported by Benito et al. (2013) and Arrobas et al. (2014) in grapes. Our results are consistent of previous findings in grapes of dilution effect in response to rapid leaf expansion and nutrient translocation from shoot to leaves and fruits. After bloom P rapidly accumulated in fruits, leaves and shoots

Table 6
Dry matter and total macro-nutrient amount (mean ± SD) in various plant parts of pomegranate tree at harvest time.

Plant part	Dry matter (Mg ha^{-1})	N kg ha^{-1}	P	K	Ca	Mg	S
Leaves	1.07 ± 0.02^{f}	14.74 ± 0.71^{c}	1.51 ± 0.15^{c}	14.51 ± 0.38^{b}	30.05 ± 1.47^{c}	2.69 ± 0.21^{c}	0.60 ± 0.16^{d}
Shoots	1.55 ± 0.02^{e}	10.23 ± 0.89^{e}	1.55 ± 0.28^{c}	7.50 ± 0.45^{d}	22.74 ± 1.02^{d}	3.22 ± 0.29^{c}	5.75 ± 0.52^{a}
Fruits	2.61 ± 0.11^{a}	20.15 ± 1.68^{a}	3.33 ± 0.35^{a}	32.18 ± 1.87^{a}	11.78 ± 1.15^{e}	7.92 ± 0.91^{a}	2.24 ± 0.50^{c}
Branches	2.01 ± 0.04^{c}	12.47 ± 1.10^{d}	1.13 ± 0.30^{d}	7.08 ± 0.89^{d}	38.82 ± 3.06^{a}	4.10 ± 0.56^{b}	4.67 ± 0.75^{b}
Trunk	1.77 ± 0.02^{d}	8.13 ± 0.85^{f}	1.03 ± 0.27^{d}	6.61 ± 0.31^{d}	30.69 ± 0.87^{c}	4.26 ± 0.66^{b}	4.94 ± 0.50^{b}
Roots	2.49 ± 0.02^{b}	18.87 ± 0.95^{b}	2.52 ± 0.37^{b}	11.09 ± 0.61^{c}	33.21 ± 2.22^{b}	7.97 ± 1.04^{a}	5.75 ± 0.81^{a}
Total	11.51 ± 0.08	84.58 ± 2.77	11.07 ± 1.01	78.96 ± 3.18	167.28 ± 5.06	30.15 ± 2.82	23.96 ± 1.73

*Results within a column are significantly different (LSD at $P < 0.05$) when letters following the value differ.

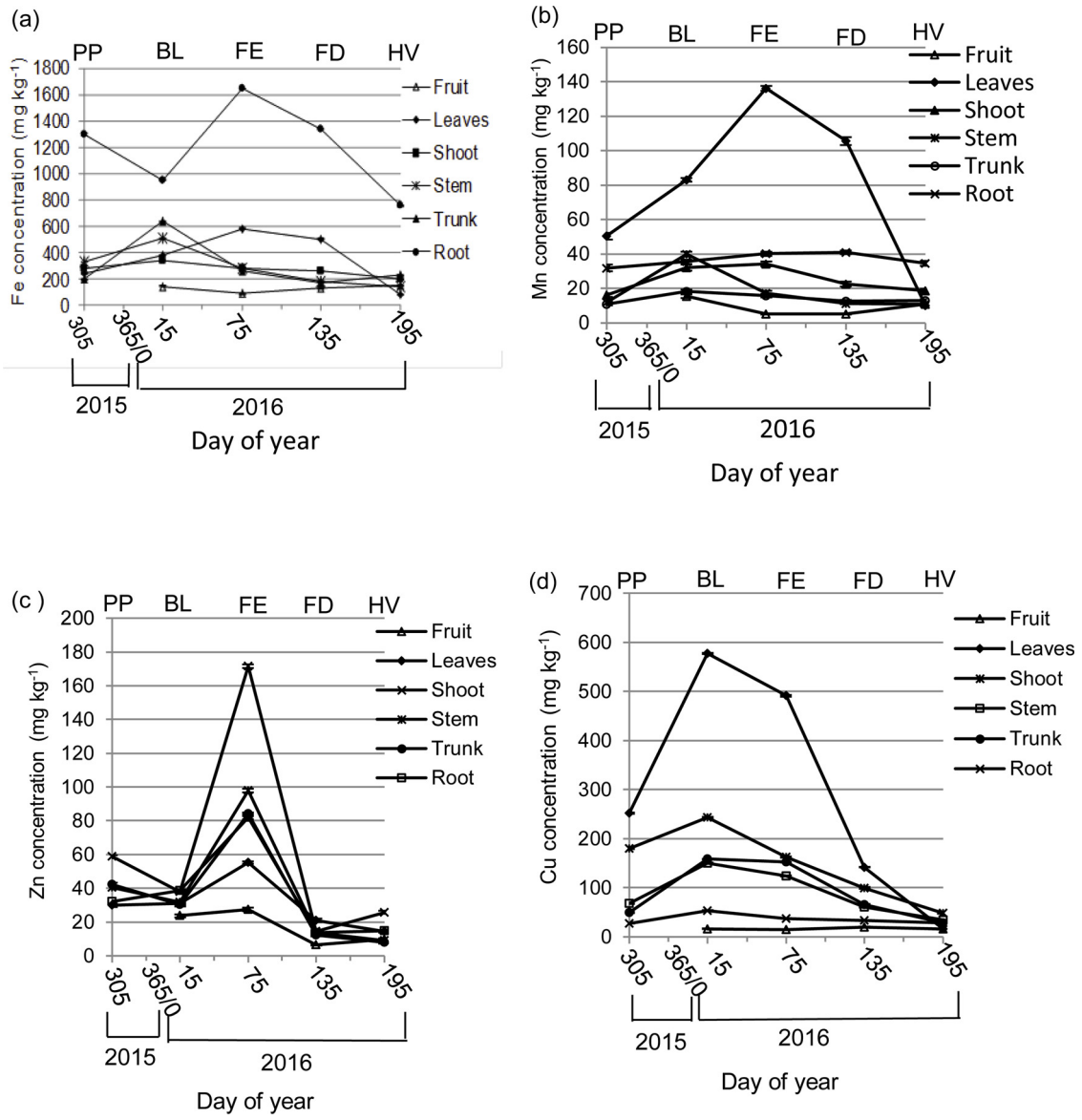

Fig. 8. Seasonal change in concentration of (a) iron (b) manganese (c) zinc and (d) copper in various organs of 'Bhagwa' pomegranate. Demarcations at the top of graph indicate the time of pre-pruning (PP), bloom (BL), fruit enlargement (FE), fruit development (FD) and harvest (HV). SE are represented as bars above and below the mean.

during fruit enlargement stage. Phosphorus that was remobilized to the canopy from stored reserve came predominantly from the large woody roots owing to low supply as indicated by low P concentration in fruits at harvest. Schreiner et al., (2016) reported similar remobilization of P from roots to support canopy demand in Pinot noir grapes. However, Conradie (1981) found little remobilization of P from roots to support canopy demand, most likely because P was in relatively high supply and vines were much younger in that study. The uptake of P during fruit development period was least owing to dry soil condition prevailing during this period (April-May) and less demand of canopy. As the soil condition improved with the availability of moisture during fruit maturity period (June – August), P uptake again increased and majority uptake of P (47.18%) took place during this period. However, the woody plant parts, branches and trunk showed minimum P concentrations at harvest. These results seem to indicate possible remobilization of P to roots occurred earlier in this period in comparison to the remobilization of N (Arrobas et al., 2014). This led to build up of P amount in the root during pre-pruning phase and subsequently a large drop in P amount in woody organs was recorded during pre-pruning phase to bloom. The decrease of P concentration during this period suggested that the new growth of pomegranate depends on the nutrients stored in permanent structures. Similar behavior of P was also observed by Pradubsuk and Davenport (2010) during bud break to three to four leaf stage in grape.

The amount of K in developing fruits increased substantially until harvest. This implies that K is most closely related to fruit development and production. The strong demand of fruit for K was evident in our study as observed in previous studies with grapevine (Schreiner and Scagel, 2006; Williams and Biscay, 1991; Williams, 1987). The movement of K into the fruit was maintained at high rate from bloom to fruit enlargement stage and again during fruit maturity stage. Similar accumulation of K in fruits was reported by Schreiner and Scagel (2006); Rogiers et al. (2006) and Schaller et al. (1992). Further, more prevailing accumulation during fruit enlargement stage supported a relative role of K in cell expansion (Rogiers et al., 2006). Very little K was remobilized to the developing canopy of pomegranate from stored reserves in the trunk and roots. Overall canopy demand and whole plant uptake for K were similar indicating that most of the plant K was

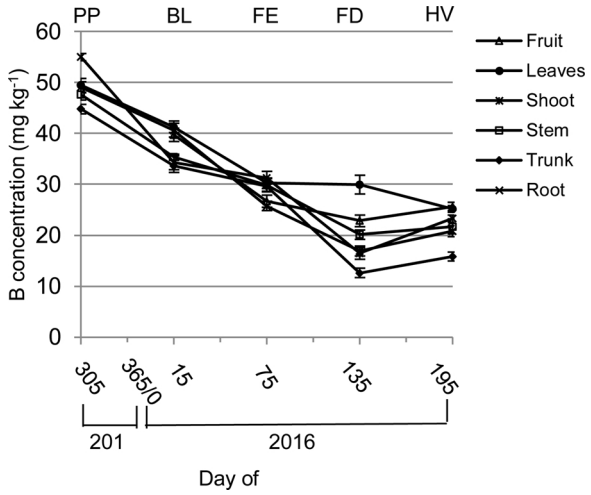

Fig. 9. Seasonal change in concentration of boron in various organs of 'Bhagwa' pomegranate. Demarcations at the top of graph indicate the time of pre-pruning (PP), bloom (BL), fruit enlargement (FE), fruit development (FD) and harvest (HV). SE are represented as bars above and below the mean.

supplied from soil uptake. We saw no evidence of remobilization of K from the leaves or branches to support fruit demand late in the season, as observed by others (Schreiner and Scagel, 2006; Williams and Biscay, 1991), rather K might have remobilized to fruits from shoot during maturity stage as seen from the drop in K amount as well as concentration in shoots. Unlike N, trunk and roots supplied small quantity of K that was remobilized during early fruit growth stage from stored reserves to the canopy.

The pomegranate crop lost 32.18 kg K ha^{-1} through removal of fruits at harvest. This amount represents ~ 3.12 kg K per tonne of fresh fruit, amounting 1.6 times that of N removal with harvest. Although, the magnitude of K removal through fruit harvest in 'Bhagwa' is higher than that (1.74 kg/tonne of fresh fruit) reported in other cultivar of pomegranate cv. 'Ganesh' (Raghupathi and Bhargava, 1996), it is far low if compared to other fruit crops such as mango (6.7 kg/ton), banana (20.3 kg/ton), citrus (11.7 kg/ton), grapes (9.0 kg/ton) and guava (7.5 kg/ton) commonly grown in this agro-eco-region. Further, if prunings are removed, the orchard may lose another ~ 8 kg K ha^{-1}. This suggests that K should be replenished after each cropping at equivalent proportion to that of N. But in the existing recommendation it is only 40% of the recommended dose of nitrogen (Firaka and Kumbhar, 2002). If necessary steps are not taken to replenish soil K in accordance with its removal, it will lead to mining of potassium from pomegranate growing soil and the long-term impact of which would be disastrous in near future. Potassium contained in the leaves will return to the soil through mineralization of senescing leaves if incorporated with the soil. Potassium does not undergo gaseous losses and the risk of K leaching from the soil is negligible than that of N. Thus, the component of recycling is easier to establish for K than N. Therefore, potassium fertilization at optimum level requires the information on the K availability in the soil and the K nutritional status of the orchard, since any excess in K application should be avoided owing to its negative impact on plant's innate defence system against bacterial blight disease (Maity et al., 2016) in contrast to numerous report in favour of K nutrition for improving disease resistance (Huber and Graham, 1999; Sharma and Duveiller, 2004; Sharma et al., 2005).

Calcium and magnesium accumulated primarily in the leaves over the fruit growth period, although Mg also accumulated in fruits. This difference is likely due to low phloem mobility of Ca versus Mg (Marschner, 1995) and greater immobilization of Ca in the form of Ca-pectate in the cell wall of maturing leaves. Hence little diversion from leaves to other plant parts took place. However, we could notice a considerable amount of Mg got remobilized from leaves to fruits and shoots during fruit maturity stage. This implies that considerable mobility of Mg take place through phloem in pomegranate. This is in contrary to the earlier reports in grape by Arrobas et al. (2014) who reported low mobility of Mg in phloem. During new leaves emergence phase after defoliation Ca is very much required by the plant as it plays an important role in maintaining the structural integrity (e.g. membrane stabilization and wood formation) of branches which would be holding flowers and fruits in the subsequent phases (Fromm, 2010). Besides, Ca is involved in protein phosphorylation and signaling in the mechanism of bud dormancy release (Pang et al., 2007). That is why, we could find majority uptake of Ca (about 61.10% of the total uptake) before blooming. At harvest fruits contained very small amount of Ca and Mg in relation of other plant parts. Calcium and Mg supplied to the canopy came largely from soil uptake and translocated through xylem (White and Broadley, 2003) with essentially none coming from stored reserves. Pomegranate plant in this study had much higher total amount of Ca than even primary nutrient like N and K throughout the growing season. And majority of pomegranate growing areas where it is grown in commercial scale are characterized by high $CaCO_3$ content of soil (Pawar et al., 2014; Narale et al., 2015). This suggests that Ca is very much essential prerequisite for successful cultivation of pomegranate.

The amounts of P, Ca and Mg removed from the system through fruit harvest are small and the nutrients contained in the leaves can be recycled in the soil. The recommendation system should involve monitoring soil fertility and plant nutritional status in order to decide if any abnormal situation exist that should be corrected and managed properly.

Sulphur concentration was higher in shoots throughout the fruit growth period indicating its intermediate mobility within the pomegranate plant. This is in the line of earlier report by Marschner (1995) who reported S as less mobile element within the plant. So, the deficiency symptoms of S are likely to appear in younger leaves unlike N. Its uptake pattern indicated that majority uptake took place before bloom and it got accumulated in roots, shoots and branches. After bloom S in roots and shoots got translocated to fruits during enlargement and development stages as S uptake by the plant was negligible during this period. Like other macro-elements S concentration in fruit declined as

Table 7
Total micro-nutrient amount (mean ± SD) in various plant parts of pomegranate tree at harvest time.

Plant part	Fe g ha^{-1}	Mn	Zn	Cu	B
Leaves	88.94 ± 6.42^{e*}	11.05 ± 2.27^{d}	15.57 ± 1.59^{cd}	17.68 ± 2.44^{e}	27.03 ± 1.38^{d}
Shoots	303.88 ± 9.13^{c}	28.65 ± 3.25^{b}	39.86 ± 3.00^{a}	74.60 ± 4.23^{a}	32.23 ± 3.94^{d}
Fruits	387.98 ± 20.58^{b}	28.53 ± 4.18^{b}	25.22 ± 4.47^{b}	40.92 ± 4.52^{d}	66.77 ± 6.40^{a}
Branches	287.97 ± 13.39^{d}	21.54 ± 2.59^{c}	18.44 ± 3.56^{c}	70.26 ± 4.85^{ab}	43.68 ± 7.01^{c}
Trunk	399.76 ± 13.94^{b}	22.66 ± 3.34^{c}	13.81 ± 2.44^{d}	49.91 ± 5.11^{c}	28.00 ± 3.79^{d}
Roots	1879.79 ± 11.44^{a}	85.85 ± 7.99^{a}	36.53 ± 3.47^{a}	69.09 ± 4.28^{b}	58.04 ± 3.93^{b}
Total	3348.32 ± 41.90	198.76 ± 14.58	149.42 ± 16.17	322.45 ± 15.57	255.75 ± 24.57

* Results within a column are significantly different (LSD at $P < 0.05$) when letters following the value differ.

the fruit grew more probably due to dilution effect as reported in tamarillo (Clark et al., 1989), Japanese pear (Buwalda and Meekings, 1990) and 'Navel'orange (Storey and Treeby, 2000). During fruit maturity stage as soil moisture increases owing to seasonal rain, the amount of S in different plant parts increased except leaves where it decreased. It may be due to remobilization and accumulation of S from leaves to roots and shoots before their senescence. Like P, Ca and Mg, amount of S removed from the system through fruit harvest is small. However, pruned shoots contain cognizable amount of S which can be recycled if pruned materials are left in the field.

4.2. Micronutrients

The overall seasonal trends in declining Fe, Mn, Zn and B are typical of fruits crops (Clark et al., 1989). This implies that accumulation rate of these nutrients are much lower than fruit biomass growing rate resulting dilution of these nutrients in fruit (Maity et al., 2017). The inflections in the declining trend indicate the period where necessary management practices are required to be adopted to maintain a good balance of nutrients for optimizing plant nutrition for improved productivity and quality. Iron concentrations in roots are several times higher than Fe concentrations in the leaves. Similar findings have been reported in grapes (Schreiner and Scagel, 2006) and *Corymbia citriodora* (Trueman et al., 2013). This shows that transport of Fe across the plasma membrane is poor. This may be due to depressed activity of Fe^{III} reductase under alkaline soil condition (pH > 7.8) (Mengel, 1994). Concentrations of Mn and Cu in leaves are several time higher than in fruits even when their concentration declined in fruits. This indicates that they have limited mobility through phloem. Classification of nutrients as phloem mobile, immobile or of intermediate mobility differs with the plant species (Marschner, 1995). Story and Treeby (2000) and Schreiner and Scagel (2006) could find Mn as phloem immobile micronutrient in navel orange and grapes respectively which rightly corroborate our observation in pomegranate. However, we could notice immobility of Cu through phloem in addition which is in contrary to their findings. Boron is the second most abundant micronutrients in fruits after Fe. The uniformity of B concentration between leaves and sinks, such as fruits present the evidence of significant movement of B within the plant via phloem. There is large range in B mobility between species which has been attributed to polyol production in source leaves and the formation of B-polyol complexes in the phloem (Brown and Shelp, 1997). Since B is mobile element in soil as well as in plant, the application should be annual after it is being noticed that the soil does not supply enough B for plant metabolism.

4.3. Conclusions

Dry weights of 4-years old pomegranate plant ranged from 3112 to 12181 g. The highest biomass of the whole plant occurred at harvest which was about 3.9 times of that at pre-pruning phase. Calcium was the most abundant mineral nutrient in the plant followed by N and K. Different organs accounted for the majority of changes in the amount of N, P, K and Ca during fruit maturity stage i.e. 121–180 DAFB. More than 60% of the Ca was in woody organs, upto 41% and 69% of total amount of K confound in fruits and annual tissues respectively. The highest accumulation of Ca in plant permanent structures reflects high Ca status in the orchard soil which is its natural habitat. Throughout the growing season, major changes in the amount of Mg occurred in fruits whereas the major changes of K took place in leaves and fruits which implies their importance in enhancing productivity. Most of the uptake of N, K, Ca, Mg and S from the soil occurred between pre-pruning phase and bloom whereas the highest P uptake took place from fruit development to fruit maturity. So, the post-harvest period is very important time for pomegranate plant to replenish its reserve of majority nutrients. The demand for micronutrients particularly Fe, Mn and B was highest during fruit enlargement stage while that of Zn during fruit development stage. Developing nutrient management practices based on these facts will be of far reaching consequences for raising productivity level of pomegranate.

Acknowledgements

The corresponding author is grateful to Indian Council of Agricultural Research (ICAR) for providing financial support and Former Director, Dr. R.K. Pal, National Research Centre on Pomegranate, Solapur for their kind cooperation.

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