

Original Research Paper

Effect of Leaf Removal on Composition of Wine Grape Varieties Grown in Semi-arid Tropical Climate of India

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ABSTRACT

Removing leaves from cluster zone is one of the management practices followed to improve fruit composition in temperate wine grape growing countries. However, knowledge on canopy management practices to improve fruit and juice composition for quality wine making is still lacking in semi-arid tropical regions of India. Due to ample sunlight availability during fruit growth in semi-arid tropics, it is unclear whether the leaves have to be removed from cluster zone. In case the leaves have to be removed, the direction from which it has to be done is also important. Hence, this study was conducted to see the effect of leaf removal from two sides of canopy on fruit composition in two wine grape varieties. In Cabernet Sauvignon vines leaf removal from both east and west side of the canopy improved fruit quality in terms of reduced pH, potassium, malic acid and increased phenolics. Nevertheless, removing leaves from eastern side was found to be better than western side, because clusters are exposed to excess sunlight. However, in Sauvignon Blanc, leaf removal from east side improved most of the desirable fruit composition parameters, while leaf removal from west side reduced the fruit quality in terms of sugars, acids, pH, total phenols etc.

Key words: Cabernet sauvignon, Sauvignon blanc, Fruit composition, Leaf removal, Organic acids, Phenolic compounds

INTRODUCTION

Though practice of wine grape cultivation is increasing in many of the tropical countries of the world, its commercial cultivation is gaining impetus only from past 50 years as compared to traditional temperate wine producing countries. Among tropical countries, Brazil, India, Venezuela etc. are playing a lead role in production of wine grapes. In last two decades, there was a substantial increase in area of grape cultivation in tropical countries. The increase was most rapid in Asian countries like India, Thailand, Myanmar and Vietnam, where new vineyards for table grape and wine production are established every year. As there is no dearth of sunlight in these areas during the annual vine growth cycle, there is a need to study the impact of sunlight exposure to clusters and high temperature on berry composition and thereby wine quality. Most of the work on canopy management practices in wine grapes is reported from UAS (Main and Morris, 2004; Bergqvist *et al.*, 2001), New

Zealand (Kemp *et al.*, 2011), Australia (Ristic *et al.*, 2013), European countries (Targaduilu *et al.*, 2008) etc. Important canopy management practices which are being practiced in most of the wine grape growing countries are shoot thinning, shoot positioning, cluster thinning and leaf removal in cluster zone etc. either alone or in combinations. These management practices help in optimizing sunlight interception, photosynthetic capacity of leaves and fruit microclimate to improve fruit composition and wine quality. Leaf removal at fruit zone in many of the cultivars showed improved fruit composition in terms of soluble solids, juice pH, phenolic compounds, anthocyanins and aroma (Kemp *et al.*, 2011). However, in some varieties there was no significant influence in quality of grapes or wines from leaf removal treatment (Reynolds *et al.*, 1986). Leaf removal at veraison stage is known to improve the fruit composition by reducing juice pH due to reduced potassium and malic acid concentration (Lang and Thorpe, 1989). The senescing leaves before falling

from vines re-translocate photosynthates into permanent vine parts. During that period, developing clusters are strong sink and hence more potassium diverts towards clusters from older leaves (Kodur, 2011). Hence, removing leaves at veraison is found to be beneficial in reducing potassium accumulation and it improves cluster exposure to sunlight improving accumulation of several beneficial secondary metabolites. Since leaf removal is one of the important management practices in improving the wine grape quality in warmer regions, it is always a question in which side of the canopy; leaves should be removed or retained to optimize cluster exposure to sunlight to harness the advantages. Hence, this study was undertaken to study the influence of leaf removal from two sides of canopy at cluster zone to know its effect on fruit composition of both red and wine grape cultivars.

MATERIALS AND METHODS

Location and plant material

This experiment was conducted at the experimental vineyard of ICAR-National Research Centre for Grapes, Pune that is located in Midwest of Maharashtra state (India) at an altitude of 559 m above the mean sea level. It lies in 18.32° N latitude and 73.51° E longitude. Five-year-old Cabernet Sauvignon and Sauvignon Blanc grapes grafted on to 110R rootstock were selected for this study. The vines were planted at a spacing of 2.5 m between rows and 1.2 m between vines within a row. The row orientation was in the direction of North - South. The vines were trained to double cordon small T system. The pruning biomass of the vines was in the range of 1.0-1.25 kg. Approximately 32 to 36 shoots were retained per vine by thinning out excess shoots.

Imposition of leaf removal treatments

Leaf removal treatments were imposed during veraison stage of berry development. On all fruit bearing shoots, leaves at cluster zone (basal five leaves and 2-3 leaves above clusters) were removed. Four different sets of variations were created on about 40 vines (10 vines per replication) as follows:

Treatment 1: Leaf removal on East side of the canopy (East LR)

Treatment 2: No leaf removal on East side of the canopy (East Control)

Treatment 3: Leaf removal on West side of the canopy (West LR)

Treatment 4: No leaf removal on West side of the canopy (West Control)

Harvesting and recording fruit composition parameters

Harvesting was done at about 110 days after pruning in Sauvignon Blanc and about 140 days in Cabernet Sauvignon varieties. After harvesting, about 250 berry samples were collected from each treatment replication wise. Half of the samples were utilized immediately for analysis of basic fruit composition parameters such as total soluble solids (TSS), titratable acidity, juice pH and Potassium content. Before analyzing these parameters, weight of 100 berries and weight of 50 seeds was recorded using electronic balance. The remaining half of the berry samples was stored in -20°C for analysis of organic acids and phenolic compounds using High Performance Liquid Chromatography (HPLC).

The fresh fruits were macerated in cheesecloth and were centrifuged and the supernatant was analysed for TSS (hand held refractometer with temperature compensated to 20°C); acidity (titration of juice against 0.1N NaOH using phenolphthalein as indicator); pH (pH meter, Model 420, Thermo Orion,) and potassium (Flame photometer, Model, PFP 7, Jenway Ltd, UK).

HPLC analysis of organic acids and phenolic compounds

The fruit samples stored in -20°C freezer were used for HPLC analysis. After removing samples from freezer, they were thawed overnight under refrigerated conditions. Later the fruits were macerated in cheesecloth; the resultant must was centrifuged and the supernatant was used for HPLC analysis.

Phenolic compounds

Chromatographic analysis of phenolic compounds was performed using the 1260 series Agilent Technologies HPLC, equipped with an inbuilt 4 channel-degassing unit, standard auto-sampler, 1260 infinity quaternary pump, Agilent 1260 infinity Diode array detector and an injector. The system was interfaced with a personal computer utilizing the Agilent EZ chrome elite software for control, data acquisition and further

analysis. A Zorbax Eclipse plus C18 column (4.6 mm x 100 mm 1.8 µm particle size.) was used. The analytical column was preceded by a C18 guard column to prevent any non-soluble residues from samples from contaminating the column. The injection volume maintained was 10µl with a flow rate of 0.80 mL/minute. The mobile phase consisted of A (0.2% acetic acid in 10% acetonitrile) - 95% and B (0.2% acetic acid in acetonitrile) - 5%. Prior to use, the solvent was filtered through vacuum filter and then sonicated for 5-10 minutes in an ultrasonic bath to remove air bubbles. The column temperature was maintained at 30°C. Peaks were determined at 280 nm for all the phenolic compounds.

Organic acids

The analysis of organic acids (Tartaric acid and malic acid) was done with Agilent technologies 1260 series HPLC system with Diode array detector (DAD) at wavelength of 214 nm and bandwidth of 4.0. The column used was Agilent Zorbax eclipse plus C 18 (4.6 ×100 mm 5µm). The separation was done with mobile phase of A - 95% Acidified water with orthophosphoric acid (pH 2.0) and B - 5% absolute methanol with flow rate of 0.8ml/min. Column temperature was 25° C. The injection volume was 10µl and total run time was 7 minutes.

Statistical analysis

The experiment was conducted in randomized block design with four replications and the data was analysed using SAS Version 9.3. Tukey's test was used for comparing treatment means.

RESULTS AND DISCUSSION

Leaf removal and its influence on fruit composition

The influence of leaf removal from two different sides of canopy on basic fruit composition parameters in Cabernet Sauvignon is given in Table 1. Leaf removal from both east and west side of the canopy has reduced the berry weight compared to their control counterparts. The maximum 100-berry weight of 100.60 g was recorded in west control vines followed by east control vines. Leaf removal from east side of the canopy has recorded minimum berry weight of 95.2 g. There was no significant difference among the treatments for seed weight. The maximum total

soluble solids (TSS) of 22.43°B and lowest acidity (0.53%) were recorded in vines with east leaf removal treatment, while west control recorded the least TSS on vines. Significant difference was recorded for potassium content with highest in east control vines (1748 ppm) and lowest in west leaf removal (1570 ppm) vines. Similarly highest juice pH was recorded with east control vines (3.58) and least with west leaf removal vines (3.43). There were no significant differences among treatments for anthocyanin and malic acid content.

The fruit composition parameters of Sauvignon Blanc grape in relation to leaf removal treatments from different canopy sides are presented in Table 2. The highest berry weight was recorded in vines which received east leaf removal treatment (104.40 g) followed by those on west leaf removal vines (101.60 g). Significant differences were recorded for TSS, acidity, pH, potassium, tartaric and malic acid content. Highest TSS (22.62 °B) and lowest acidity (0.50%) were recorded on vines with east leaf removal vines. Both east and west control vines recorded higher values for potassium (1782 and 1658 ppm) than leaf removal treatments. The lowest juice pH was recorded with east leaf removal treatment (3.46), while it was highest in east control vines (3.62). Highest malic acid was recorded in east control (3.87 g/L) followed by west control (3.78 g/L) vines, while it was lowest in east leaf removal vines (2.89 g/L). Sunlight intensity received at different zones in the vine canopy is known to strongly influence fruit composition such as sugars, acids, and other secondary metabolites involved in wine aroma including phenolics (Downey *et al.*, 2006).

Accordingly, many viticultural treatments associated with canopy management are intended to manipulate photosynthetic photon flux (PPF) of the fruiting zone or the distribution of photon flux across the total leaf area of the canopy to achieve metabolic effects. In grapevines, depending on cultivars and canopy management practices, leaves and bunches can develop in zones varying from heavily shaded to fully exposed clusters. Generally, the berries that develop in open canopies have high sugar concentrations, improved acid metabolism and increased concentrations of berry phenolics including anthocyanins (Gladstone, 1992).

The sunlight exposure to clusters through these canopy management practices to obtain good quality fruits varies with variety to variety. In the present study, leaf removal on both the sides of the canopy in Cabernet Sauvignon vines resulted in reduced berry weight and increased TSS compared to control vines. However, in Sauvignon Blanc, there was increase in berry weight on leaf-removed vines compared to control vines. This variation in berry weight among different varieties is in agreement to the previous findings of reduced berry weight in clusters developed in shaded part of canopy compared to that of exposed clusters. The reduced berry weight in Cabernet Sauvignon might be due to elevated berry temperature through more exposure of clusters in leaf-removed vines resulting in reduced berry cell division and elongation coupled with increased berry transpiration and consequent berry dehydration (Bergqvist *et al.*, 2001).

Many investigators found that sunlight exposed fruits are generally rich in total soluble solids and reduced titratable acidity compared to non-exposed or shaded canopy (Ferree *et al.*, 2004; Main and Morris, 2004). But, in contrast some workers found that defoliation had no effect on soluble solids and titratable acidity (Vasconcelos and Castagnoli, 2000; Poni *et al.*, 2006). The decline in titratable acidity with increased exposure to sunlight may be attributed to increased malic acid degradation due to the higher temperatures of exposed fruit (Lakso and Kliewer, 1978). Though acidity was highest on grapes harvested from control vines, the juice pH was also highest on those vines, while it was least on leaf-removed vines. The pH of grape juice or wine usually results from the balance between anionic forms of organic acids (mainly malic acid and tartaric acid) and the major cations such as potassium (Boulton, 1980). It is very well established concept that juice pH in grapes is determined by concentration of juice potassium and malic acid (Kodur *et al.*, 2010; Kodur, 2011). In this study, leaf removal treatments in both the varieties recorded least juice pH and lower concentrations of malic acid and potassium content compared to control vines.

Leaf removal at veraison stage affects synthesis of primary and secondary metabolites and this effect is directly related to leaf layer number, photosynthetic rate and canopy surface area. Several experiments have shown increased sugars, flavor, flavonoids and

decreased acidity when leaf removal was done at veraison stage (Poni *et al.*, 2006). In contrast, leaf removal at veraison on plants with low canopy density does not affect grape sugar, acidity, or color (Reynolds *et al.*, 1986). In this study, the leaves were removed from cluster zone at the time of veraison in both the varieties. It is observed that at the time of veraison, most of the potassium accumulated in leaves get diverted towards developing berries leading to increased potassium content in berries (Lang and Thorpe, 1989). Similarly, clusters developed in shaded portion of the canopy are known to accumulate more malic acid than open canopies. The increase concentration of malic acid in control berries may be due to reduced metabolic rate of malate degradation, which was otherwise used as respiratory substrate in post veraison berries (Morrison and Noble, 1990). Thus, leaf removal at cluster zone during veraison had dual advantages of reducing the potassium translocation from older leaves into clusters and reduced malic acid accumulation. Kliewer and Smart (1989), also recorded more potassium, malic acid in shaded berries than fully exposed berries.

Leaf removal and its influence on phenolic profile

The influence of leaf removal on phenolic profile of Cabernet Sauvignon grapes is presented in Table 3. Among flavan-3-ols, there was increase in catechin and epicatechin content in vines, which received leaf removal treatment on both the sides, compared to their control counterparts. Highest catechin content was recorded in east leaf removal vines followed by west leaf removal vines. There was significant increase in quercetin content from control vines to leaf removed vines. Vines with west leaf removal treatment recorded maximum quercetin content (13.12 mg/L) while, it was minimum in east control (6.61 mg/L). Most of the non-flavonoid phenolic compounds such as gallic acid, vanillic acid, coumaric acid and chlorogenic acids were increased with leaf removal treatment compared to control treatments. The concentration of these phenols was in the order of gallic acid > coumaric acid > chlorogenic acid > caffeic acid > vanillic acid. Most of these compounds were highest in East leaf removal treated vines than in west leaf removal treated vines except coumaric acid, which was highest in west leaf removal treated vines (3.80 mg/L). Piceatanol, a stilbene was highest in west leaf removal treated vines (39.41 mg/

L) followed by west control vines (30.97 mg/L). The resveratrol was detected in very trace amounts and was highest in east control vines (0.056 mg/L). The total phenolic compound was significantly highest in west leaf removal vines (94.01 mg/L) compared to east leaf removal vines (72.09 mg/L).

The influence of leaf removal from different sides of canopy on phenolic profile of Sauvignon Blanc grapes is given in the Table 4. Among flavan – 3 - ols, catechin and epicatechin accounted for maximum concentration followed by quercetin and myrecetin (both flavonols). Highest catechin content was recorded in East leaf removal (16.77 mg/L) followed by west control (14.86) vines. Most of the non-flavonoid contents were increased in east leaf removal vines as compared to east control vines, while it was decreased in west leaf removal treated vines compared to west control vines. The concentrations of non-flavonoid phenolic compounds in east leaf removal treated vines were on par with west control vines, while it was minimum in west leaf removal vines. The total phenolic content was highest in east leaf removal vines (43.11 mg/L) followed by west control vines (37.37 mg/L), while minimum total phenolic compound was recorded in west leaf removal vines (30.39 mg/L).

Though there was no significant difference among treatments in anthocyanin concentration in Cabernet Sauvignon grapes, there was slight reduction in its accumulation in control vines. In control vines, it is likely that light is a limiting factor for anthocyanin accumulation. Many authors have confirmed that shading reduces anthocyanins accumulation (Dokoozlian and Kliewer, 1996; Jeong *et al.*, 2004). Similarly, it was opined that anthocyanin synthesis is directly regulated by both the light exposure and the temperature conditions to which a grape bunch is subjected (Smart *et al.*, 1988). Management practices that create a canopy architecture where bunches receive sufficient light for anthocyanin synthesis, but berries are protected from excessive berry heating, would seem appropriate for the production of fruit with optimal levels of anthocyanins for vines grown in hot climates. Based on the previously established scientific reports, Haselgrove *et al.* (2000) suggested that a desirable canopy for vines grown in hot climatic conditions is one where bunches are moderately exposed. Cluster shading resulted in a substantial

reduction in accumulation of flavonols and skin proanthocyanidins and minimal differences in anthocyanins in 'Pinot Noir' grapes (Cortell and Kennedy, 2006). The fruit composition with respect to juice pH, potassium and malic acid in Cabernet Sauvignon grapes with east leaf removal and west control vines are quite comparable. Hence, one should take decision whether to remove leaves from west side of the canopy as it may expose clusters to direct afternoon sunlight as it is undesirable to expose clusters to excess sunlight in hot climate as suggested by Haselgrove *et al.* (2000).

Leaf removal on different sides of canopy showed different pattern of phenolic profiles in two of the varieties studied. In Cabernet Sauvignon, leaf exposure on both east and west side of the canopy recorded increased phenolic content though percent increase was more (123%) when leaves were removed from west side compared to control vines. In variety Sauvignon Blanc, there was about 129% increase in total phenolic compounds when leaves were removed in east side of the canopy compared to control, but when leaves were removed from west side of the canopy there was about 81% reduction in total phenolic compounds compared to control vines (Figure 1). Most of the different classes of phenolic compounds increased in response to leaf removal treatment on both east and west side of the canopy in Cabernet Sauvignon variety, while in Sauvignon Blanc, increased concentration of different phenolic compounds was observed only when leaves were removed from east side of the canopy. There was reduction in all the phenolic compounds when leaves were removed from west side of the canopy compared to control vines. The increased concentration of phenolic compounds in Cabernet Sauvignon vines in response to leaf removal is in accordance to the findings of Ristic *et al.* (2007), wherein shaded clusters of Shiraz could accumulate only traces of quercetin compared to those in exposed clusters. This clearly suggests the role of leaf removal to expose clusters to sunlight for accumulation of quercetin is a light dependent process in colored varieties. However, when clusters were exposed on west side of the canopy in Sauvignon Blanc, there was reduction in Quercetin content. Among stilbenes, content maximum concentration of piceatannol was recorded in both the varieties compared to resveratrol. Leaf removal from both sides of canopy in Cabernet

sauvignon increased piceatannol content, but it was reduced in Sauvignon Blanc. The increased concentration of piceatannol compared to resveratrol might be because it is glucosylated resveratrol metabolite as opined by Waterhouse and Lamuela-Raventos (1994). Though it is said that colored grapes have high concentration of stilbene, some studies could not establish as they could determine higher concentration of stilbene (trans resveratrol, piceid etc.) in some white grape cultivars compared to those in red grape cultivars (Mikes *et al.*, 2008).

CONCLUSIONS

Practice of removing leaves from different sides of vine canopy may not be uniform for all grape varieties grown in a given soil and climatic conditions. In this preliminary study, significant variation in fruit composition parameters was observed due to leaf removal from different sides of vine canopy in both the varieties. Cabernet Sauvignon being red variety could produce fruits with lower pH, high TSS; lower potassium and malic acid content and higher concentration of anthocyanin, when leaves were

removed from east side of the canopy. However, better fruit quality with respect to juice pH, potassium and malic content were comparable between east leaf removal and west control vines.

In Sauvignon Blanc, leaf removal on west side of the canopy resulted in drastic reduction of phenolic compounds compared to leaf removal from east side of the canopy. In this particular variety, leaf removal from east side of the canopy was found to be better. Hence, leaf removal should be carried out selectively in different sides of the canopy depending on the vigor of the variety. In both the varieties, leaf removal on east side of the canopy could produce quality grapes measured in terms of lower juice pH coupled with reduced potassium and malic acid. A high degree of leaf removal to expose bunches in west side of the canopy under tropical climatic conditions may not be desirable for obtaining good quality wine grapes. Further studies on degree of variation in light intensity and berry temperature under the influence of leaf removal will help in better understanding of fruit chemistry on leaf-removed vines.



Fig. 1: Effect of leaf removal on percent change in phenolic contents in comparison to control vines

Table 1: Effect of leaf removal on fruit composition of Cabernet Sauvignon grapes

Treatment	Berry wt (g)	Seed wt (g)	TSS (°B)	Acidity (%)	Juice pH	Potassium (ppm)	Anthocyanin (g/mg)	Tartaric acid (g/L)	Malic acid (g/L)
East Control	100.00	1.43	21.37	0.59	3.58	1748	0.83	6.50	3.84
East LR	95.20	1.45	22.43	0.53	3.43	1648	1.00	5.04	3.51
West Control	100.60	1.47	21.31	0.61	3.52	1636	0.80	6.52	3.92
West LR	95.60	1.46	21.86	0.56	3.56	1570	0.84	6.26	3.62
SEM ±	1.59	0.015	0.32	0.01	0.041	33.06	0.12	0.225	0.22
CD@ 5%	3.39	0.032	0.69	0.03	0.087	70.46	0.26	0.479	0.47
Pd ^{0.05}	0.049*	NS	NS	0.004	0.044	0.012	NS	0.023	NS

*: Values below 0.05 are statistically significant at Pd^{0.05}; NS: Non significant

Table 2: Effect of leaf removal on fruit composition of Sauvignon Blanc grapes

Treatment	Berry wt (g)	Seed wt (g)	TSS (°B)	Acidity (%)	Juice pH	Potassium (ppm)	Tartaric (g/L)	Malic (g/L)
East Control	96.39	1.43	21.26	0.606	3.62	1782	7.40	3.87
East LR	104.40	1.46	22.62	0.500	3.46	1588	6.14	2.89
West Control	98.60	1.46	21.88	0.622	3.61	1658	7.15	3.78
West LR	101.60	1.46	21.62	0.582	3.52	1650	6.66	3.21
SEM ±	1.953	0.017	0.268	0.016	0.039	29.46	0.239	0.208
CD@ 5%	4.161	0.036	0.571	0.034	0.083	62.77	0.509	0.443
Pd ^{0.05}	0.049	NS	0.016	0.0003	0.030	0.002	0.002	0.001

*: Values below 0.05 are statistically significant at P=0.05; NS: Non significant

Table 3: Effect of leaf removal on phenolic profile (mg/L) of Cabernet Sauvignon grapes

Rootstocks	Flavonoid phenolic compounds					Non flavonoid phenolic compounds					Total			
	Flavonols and Flavonol aglycons		Hydroxy benzoic acids			Hydroxy cinnamates		Stilbene						
	Flavan -3-ols	Quercetin in catechin	Rutin hydrate	Kaempferol	Myricetin	Gallic acid	Vanillic acid	Caffeoyl acid	Chlorogenic acid	Resveratrol		Piceatannol		
East Control	20.78	7.11	6.61	0.027	0.23	0.000	3.48	0.21	2.07	1.39	2.05	0.056	17.26	62.65
East LR	24.89	6.80	10.56	0.030	0.37	0.015	3.92	0.57	2.20	2.16	2.87	0.026	17.34	72.09
West Control	20.98	5.62	7.78	0.041	0.13	0.001	3.20	0.11	0.97	2.29	1.77	0.033	30.97	76.01
West LR	22.30	5.87	13.12	0.036	0.11	0.000	3.89	0.40	0.80	3.80	1.68	0.025	39.41	94.01
SEM±	2.39	1.07	0.803	0.006	0.033	0.003	0.30	0.12	0.48	0.396	0.290	0.023	1.432	5.034
CD@ 5%	5.09	2.28	1.711	0.012	0.070	0.006	0.64	0.26	1.03	0.843	0.617	0.049	3.051	10.424
Pd*0.05	NS	NS	0.0001	NS	0.0002	0.010	NS	NS	NS	0.004	0.04	NS	0.001	0.0036

*. Values below 0.05 are statistically significant at Pd*0.05; NS: Non significant

Table 4: Effect of leaf removal on phenolic profile (mg/L) of Sauvignon Blanc grapes

Rootstocks	Flavonoid phenolic compounds					Non flavonoid phenolic compounds					Total			
	Flavonols and Flavonol aglycons		Hydroxy benzoic acids			Hydroxy cinnamates		Stilbene						
	Flavan -3-ols	Quercetin in catechin	Rutin hydrate	Kaempferol	Myricetin	Gallic acid	Vanillic acid	Caffeoyl acid	Chlorogenic acid	Resveratrol		Piceatannol		
East Control	13.95	8.30	4.06	0.50	0.36	1.51	0.77	0.13	0.82	0.05	1.26	0.39	1.27	33.38
East LR	16.77	8.65	5.91	1.23	0.80	2.51	0.84	0.14	0.62	0.26	1.53	0.27	0.89	43.11
West Control	14.86	8.99	5.33	0.35	0.18	2.04	0.80	0.16	0.63	0.15	1.59	0.16	1.50	37.37
West LR	11.71	6.63	4.60	0.89	0.15	1.33	0.79	0.11	0.87	0.05	1.34	0.20	1.34	30.39
SEM±	1.021	0.502	0.470	0.25	0.175	0.248	0.066	0.034	0.240	0.018	0.138	0.117	0.244	1.648
CD@ 5%	2.175	1.069	1.001	0.532	0.372	0.528	0.140	0.072	0.511	0.038	0.294	0.249	0.477	3.511
Pd*0.05	0.022	0.024	0.063	0.001	0.063	0.016	0.710	0.024	0.835	0.001	0.332	0.548	0.375	0.0003

*. Values below 0.05 are statistically significant at Pd*0.05; NS: Non significant

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