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DIFFERENTIAL RESPONSE OF CITRUS GERMPLASM FOR VARYING CONCENTRATION OF ALUMINIUM

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ABSTRACT

Acidic soils with high amount of aluminium are the major problems of hilly regions of India. A study was conducted to evaluate the effect of aluminium concentrations on the growth parameters *viz*. root growth, root volume, shoot growth, dry weight of citrus germplasm. Fourteen germplasm of citrus were grown in hydroponics in Hoagland's solution having different concentrations of aluminium with 0, 50, 100, 200 and 400 µmolL^{-1} of $Al_2 (SO_4)_3.18H_2O$. The results revealed that there was general reduction in all the growth parameters observed. In general, there was a difference of response in all the germplasm tested. Among the various growth parameters, the root length and root volume were reduced markedly by the increase in concentration of aluminium. Pani Jyamir (G_{10}) and Citron (G_{14}) can tolerate higher amount of Al toxicity which can be use as root stock or can be cultivated under Al toxic conditions and that of Nagpur mandarin (G_5) was with highest reduction. Further Principal component analysis and cluster analysis were also used for determining the susceptible and tolerant germplasm for aluminium toxicity and also to group germplasm based on their tolerance on various Al concentrations.
***Keywords*:** Acidic soil, aluminium toxicity, growth parameters, citrus germplasm, Hydroponics, multivariate analysis, screening, acidic soil.

Introduction

Acidic soils with high amount of aluminium are the major problems of hilly regions of India. Plants which are grown in acid soils, due to aluminium solubility at low pH have under developed root system and exhibit a variety of nutrient deficiency symptoms consequently decrease in yield. Al interferes with nutrients uptake, transport and utilization of essential nutrients including Ca, Mg, K, P, Cu, Fe, Mn and Zn (Foy, 1984; Guo *et al*., 2003). Making of successful citrus plantation in acidic soil will be mainly depend upon identifying the germplasms which are tolerant for aluminium toxicity either to be used as cultivar or to be used as rootstocks. Since India has large number of variation in citrus germplasm screening them for aluminium toxicity will be a better option. Hence, a study was conducted to test the effect of varying concentrations of aluminium on the growth parameters of different citrus germplasm.

Materials and Methods

The research was carried out at green house of Department of Horticulture, Sikkim University, Gangtok, during the period of five months i.e., March-December 2014 at an elevation of 1230 m above M.S.L with latitude and longitude of N 27'18.495' and E 88' 35. 307' respectively. The fruits were obtained from Sikkim i.e., from Majhitar Horticulture Farm, KVK East, Martam Nazitam and outside Sikkim i.e., from Nagpur, Maharastra and Pasighat, Arunachal Pradesh which were tagged separately and were washed, demucilaged and then seeds were extracted using running water. The seeds were dried separately in room temperature for a day and were kept in a separate folded paper bag.

Seeds were sown in germination sand within a week of extraction in a utility tray of size 36.83cm X 25.4cm with 14 varieties @ 10 seeds per variety in each tray. They were sprayed with Hoagland's solution and distilled water till the germination and transplantation. After germination the germplasms were transplanted to hydroponic system in the trays containing 4 litre of double distilled water and one litre of Hoagland solution which was modified according to Simon *et al.* (1994). The seedlings were transplanted on 56th day from sowing. All the germplasms were transplanted to five different trays along with their replications.

Each tray containing Hoagland's solution and aluminium was aerated by means of air compressor. Macronutrients were added as $PO4^{2-}$ (KH_2PO_4), 3.38 $cmol_c\ kg^{-1}$., K^+ (KNO_3), 9,00 $cmol_c\ kg^{-1}$., Ca^{2+} [$Ca(NO_3)_2.H_2O$],10.13 $cmol_c\ kg^{-1}$., Mg^{2+} ($MgSO_4$. $7H_2O$), 3.38 $cmol_c\ kg^{-1}$ while the micronutrients were added as Fe^{2+} ($FeCl_3\ 6H_2O$), 1.6 mgL^{-1}., Na^+ (Na_2 EDTA.H_2O)., 1.43 mgL^{-1}., Mn^{3+} ($MnSO_4$. $4H_2O$)., 0.25 mg L^{-1}., MoO_4 [$(NH_4)6Mo_7O_{24}$. $4H_2O$],0.006 mgL^{-1}., B (H_3BO_3),0.37 mgL^{-1}., Zn^{2+} ($ZnSO_4$. $7H_2O$) ,0.12mgL^{-1} and Cu^{2+} ($CuSO_4.5H_2O$), 0.03 mgL^{-1}.

After seven days of seedling establishment in the trays, each tray was added five different concentrations of aluminium as 0, 50, 100, 200 and 400 µmol L^{-1}. The pH of the solution was monitored daily and maintained at 4.0 by adding 0.1 mol L^{-1} HCl or NaOH solutions, as necessary. The solution was changed in every two weeks.

After 60 days of growth the germplasms were removed from hydroponics system and subjected to the growth parameters analysis. Root and shoot measurement as described by Lin and Myhre, 1991, entire plant dry weight as per Pereira *et al.* (2003) and root volume by water displacement method was carried out. The root and shoot length were measured in centimetre scale. The experimental design was carried with Factorial Completely Randomized Design (CRD).

Each concentration of aluminium was treated as one treatment (Factor-1). Under each treatment fourteen germplasms (Factor-2) were screened and for each germplasm three replications were used. Statistical analysis was carried out by one-way ANOVA (analysis of variance) to compare the significance of difference between the treatments and germplasms.

Results and Discussion

Morphological characters

Plant height (cm)

Plant height of *Citrus sps.* was measured in five weeks intervals. It was found to be significantly different for all the germplasms, aluminium concentration in different levels and their interaction effect. G_1 was found to be the tallest among all the germplasm under various aluminium concentrations in fifth, fourth, third, second and first week of growth (table). In addition, fifth, fourth, second and first week of planting the growth declined at A_{50}. Whereas, fifth, fourth, second and 1st week of planting at A_{100} the height increased slowly.

In this study it was analysed that at A_{400} (highest aluminium concentration), the height of plant in 1st, third, fourth and fifth week of planting except at 2nd week where there was slight decline (table 3). The interaction effect between genotype and aluminium level showed highest reduction plant height at fifth, fourth and second week of planting.

Root length (cm)

Among all the fourteen-germplasm tested, Nagpur mandarin showed highest reduction of root growth. On the other hand, increase of root growth was observed in three different germplasms namely Rangpur lime (G_9), Citron (G_{14}) and Pani Jyamir (G_{10}). Infact Rangpur lime (G_9) showed significant increase in root length.

From the root length analysis, it was found that Rangpur lime (G_9) was highly tolerant to Al toxicity followed by citron (G_{14}), Pani Jyamir (G_{10}), Sour orange (G_6), Mosambi (G_7), Pomelo (G_{12}) and Sikkim mandarin (G_2). The highly susceptible species were Nagpur mandarin (G_5) followed by Indian mandarin (G_1), Grape fruit (G_{11}), Cleopatra mandarin (G_8), Rough lemon (G_3) and Acid lime (G_4) as illustrated in Table 3 and Fig. 1a, 1b and 1c

This trend was similar to the earlier report by Yokomizo and Ishihara (1973) in Natsudaidai seedlings; Lin and Myhre (1991) in Carrizo citrange, Cleopatra mandarin, rough lemon, Sour orange and swingle citrumelo; Santos *et al.* (1999) in Rangpur lime and swingle citrumelo; Pereira *et al.* (2003) in rough lemon and Cleopatra mandarin and Batista *et al.* (2013) in corn plants who mentioned significant reduction in root length with increasing Al concentrations.

Volume of root (cc)

The result presented on aspect of volume of root showed decreasing trend with increase in concentration of aluminium in several germplasms (Table 4 and Fig. 2.) Highest reduction was in Nagpur mandarin (G_5) whereas increase in volume of root compared to control was found in Sikkim mandarin (G_2), Rough lemon (G_3), Acid lime (G_4), Sour orange (G_6), Mosambi (G_7) and Citron (G_{14}). However, among the germplasm, Mosambi (G_7) showed highest significant increase in root volume compared to control, which is reported for the first time in present study.

From the root volume analysis, it was evident that *Citrus sinensis* (G_7) was found most tolerant to root volume followed by Sour orange (G_6) and Acid lime (G_4). On the other hand, highly, susceptible species were: Nagpur mandarin (G_5), Cleopatra mandarin (G_8), Pomelo (G_{12}) and Kala Jyamir (G_{12}).

The trend was in corroboration with the findings of Santos *et al.* (1999a) who mentioned that increase in aluminium concentration reduces the root volume showing inverse relationship of root volume and aluminium concentration. Their study revealed Swingle citrumelo and Rangpur lime with reduction in root volume at 7.5 mgL^{-1}. Whereas in Cravo lemon there was no significant influence in the root volume at this concentration. However, at 30 mgL^{-1} the decrease was observed.

Shoot length (cm)

In shoot length as depicted in Table 5 and Fig. 3, it was observed that several germplasms had shown reduction compared to control. Decrease in shoot length was recorded in eleven germplasm and that of increase in three germplasms which were significantly higher than control. Nagpur mandarin (G_5) showed highest reduction. On the other hand, increase in shoot height was found in three different germplasms *viz:* Pani Jyamir (G_{10}), Pomelo (G_{12}) and Bimira (G_{14}). In addition, Pani Jyamir (G_{10}) was among the fourteen germplasm which showed the best result in terms of tolerance to Al stress level and that of Nagpur mandarin (G_5) was observed with susceptibility.

Based on the shoot length analysis on Al stress condition, The sequential order of germplasm for Al tolerance can be Pani Jyamir (G_{10})>Pomelo (G_{12})> Citron (G_{14})>Rangpur lime (G_9)>Kala Jyamir (G_{13})>Sour orange (G_6)>Sikkim mandarin(G_2)> Cleopatra mandarin (G_8), Mosambi(G_7), Indian mandarin (G_1)> Grape fruit (G_{11})> Rough lemon (G_3)> Acid lime (G_4)> Nagpur mandarin (G_5). The report is presented first time in this study.

Dry weight of plant (g)

Dry weight of plant (DWP) invariably reduced with respect to increase in aluminium stress levels. Out of the fourteen-germplasm tested reduction was found in twelve germplasm and that of increase in two germplasms compared to control. Among all the germplasm, Nagpur mandarin (G_5) showed highest reduction of dry weight of plant on the other hand increase of dry weight was in two germplasms Sikkim mandarin (G_2) and Pani Jyamir (G_{10}). Infact Sikkim mandarin (G_2) had shown significant increase in dry weight of plant which is reported for the first time in this study.

From the dry weight of plant analysis, it was found that Sikkim mandarin (G_2) was most tolerant to aluminium toxicity followed by in most to least tolerance as Pani Jyamir (G_{10}), Acid lime (G_4), Kala Jyamir (G_{13}), Rangpur lime (G_9), Mosambi (G_7), Sour orange (G_6), Pomelo (G_{12}), Rough lemon (G_3), Cleopatra mandarin (G_8), Grape fruit (G_{11}) and Indian mandarin (G_1) as mentioned in Table 6 and Fig 4.

The result obtained in the research is in consonance with the findings of Pereira *et al.* (2003). They had mentioned that increase in Al will reduce the dry matter of leaves. The similar kind of report was presented by Santos *et al.(*1999b) who mentioned reduction in dry weight of leaves, root, stem in Swingle citrumelo with increase in Al levels and that of dry weight of lemon carnation was not significantly influenced by different levels of Al. Further, Nogueira *et al. (*1989) reported in Rangpur lime, Sunki, Tangerine, tangelo Orlando, Volkamer lemon roots and leaves had shown decrease in their dry weight with increase in aluminium levels.

Principal component analysis (PCA) and Cluster analysis

Based on the PCA, at control PC1 and PC2 contribute 82.17 % of the total variance within the dataset analysed. Root length (Rl), root volume (Rv) and shoot length (Sl) showed positive value for both PC1 and PC2. Whereas, PH1, PH2, PH3 and DWP were found with positive value for PC1 and PC2 with negative value (fig. 5). The PC analysis and cluster analysis resulted in four group of Citrus germplasm based on the resistance for the various levels of aluminium.

Group first had G1 and G11 with higher values for PH1, PH2, PH3 and DWP and moderate value of Rl, Rv and Sl. Germplasm G1, G3, G4 could be grouped in another group based on the high value of Rl, Rv and Sl and showed moderate values for remaining growth response under study. Third group comprised of G2, G8, G10 and G13 with moderate values for all the growth parameters under study. The fourth group consist of G14, G6, G9 and G7. Amongst all it had lower values for all the character under study.

At, A_{50} $\mu MolL^{-1}$ grouped them into four groups by PC analysis and cluster analysis of the germplasm under study (Fig. 6). In this PC1 and PC2 contributed 87% of the total variance present in the dataset. At 50 $\mu MolL^{-1}$ µM, Rl, Rv, Sl and PH1 had positive value for PC1 and negative value for PC2 (fig.). The first group includes of G3, G10 and G4 which was found with high value for the parameters *viz:* Rl, Rv, Sl and PH1 and moderate value for characters *viz:* PH2, PH3, PH4 and DWP. Further, G1 and G5 could be considered as the second group had higher values characters for PH2, PH3, PH4 and DWP and moderate value for Rl, Rv, Sl and PH1. Likewise, germplasm, G2 grouped as the third group had moderate values for the characters under study.

At 100 $\mu MolL^{-1}$ of aluminium applied, PC1 and PC2 both contributed 83.4% of the total variance in the data set. Based on PC and cluster analysis, all the germplasm were grouped in four clusters (fig. 7). From the fig 7 it was revealed that Rl, Rv, DWP, PH1 showed positive values for both PC1 and PC2, while PH1, PH2 and Sl had positive values at PC1 and negative value at PC2. In the first group, G11, G4 and G7 had shown moderate values for all the parameters under the research.

Further germplasm, G3, G5, G9 and G13 could be placed in second group which had shown higher value for parameters like Rl, Rv, DWP and PH5 but was moderate level in PH1, PH2 and Sl. Likewise, G2, G4 and G7 was found in another group and the last group comprises of G5, G11, G12 and G6 was found with lower values for all the parameters under the research.

Moreover at 200 $\mu MolL^{-1}$ µM of aluminium level, four groups were categorised where PC1 and PC2 showed 88.9 % of the total variance of dataset. In both PC1 and PC2, parameters like Rv, DWP and PH2 showed positive response. But Rl and Sl had positive value for PC1 and negative value for PC2 accordingly (fig 8). Germplasm G3, G10 and G12 were found in one group. Rv, DWP and Ph2 had shown higher response for this group of germplasm while Rl and Sl were moderate for this group. The second group was formed from G2, G4, G7 and G13 was found with moderate values for Rv, PH2 and DWP and lower values for Rl and Sl which can be grouped in another group accordingly. Finally, the fourth group consist of G9, G8 which have lesser value for all the parameters and can be grouped into one group.

Last group (400 $\mu MolL^{-1}$ of aluminium), which was the highest dose of aluminium applied was found with 77.4% of the total variance in the dataset. Rl, Sl, DWP and PHP were the parameters which is found in G8, G9, G10, G11 and G12. In addition, PH1, PH2 and Rv were higher in G1. G2, G4 and G14 were on the third group and lastly G3, G5, G6, G7 and G13 were i the last group which had moderate of all the parameters.

Conclusion

Among the germplasm used in the experiment, Pani Jyamir and Citron were found to be tolerant and Nagpur Mandarin was found to be highly susceptible for aluminium toxicity. Pani Jyamir, a strain of *Citrus jambhiri* and Citron (*Citrus medica*) can be used a root stock for tolerance to aluminium toxicity.

Table 1 : Germplasm used for Experiment

Sl. No.	Common name	Scientific Name
G_1	Indian Mandarin	*Citrus indica*
G_2	Sikkim Mandarin	*Citrus reticulata*
G_3	Rough lemon	*Citrus jambhiri*
G_4	Acid lime	*Citrus aurantifolia*
G_5	Nagpur Mandarin	*Citrus reticulata*
G_6	Sour orange	*Citrus aurantium*
G_7	Mosambi	*Citrus sinensis*
G_8	Cleopatra mandarin	*Citrus reshni*
G_9	Rangpur lime	*Citrus limonia*
G_{10}	Pani Jyamir	*Citrus jambhiri*
G_{11}	Grape fruit (Phokse)	*Citrus paradisi*
G_{12}	Pomelo	*Citrus maxima*
G_{13}	Kala Jyamir	*Citrus jambhiri*
G_{14}	Citron(Bimira)	*Citrus medica*

Table 2 : Different concentration of aluminium

Sl. No	Treatments	Al concentration ($\mu mol\ L^{-1}$)
1	T_1	0
2	T_2	50
3	T_3	100
4	T_4	200
5	T_5	400

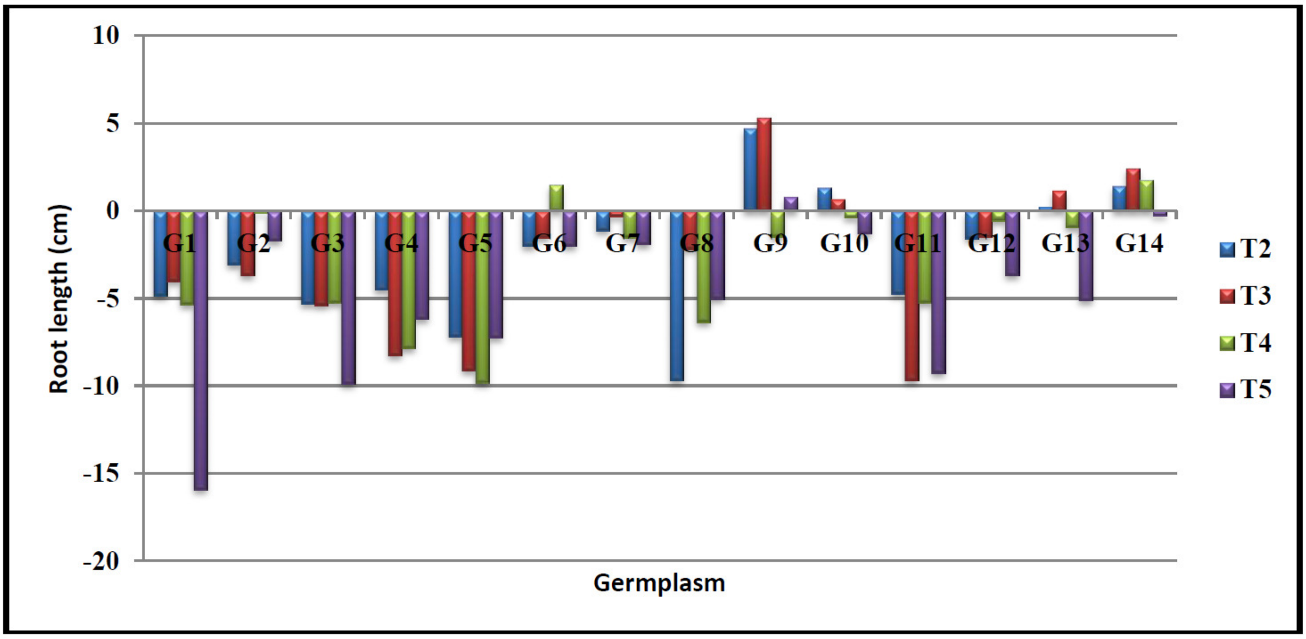

Fig 1: Reduction in root length of fourteen citrus germplasm under various aluminium concentration

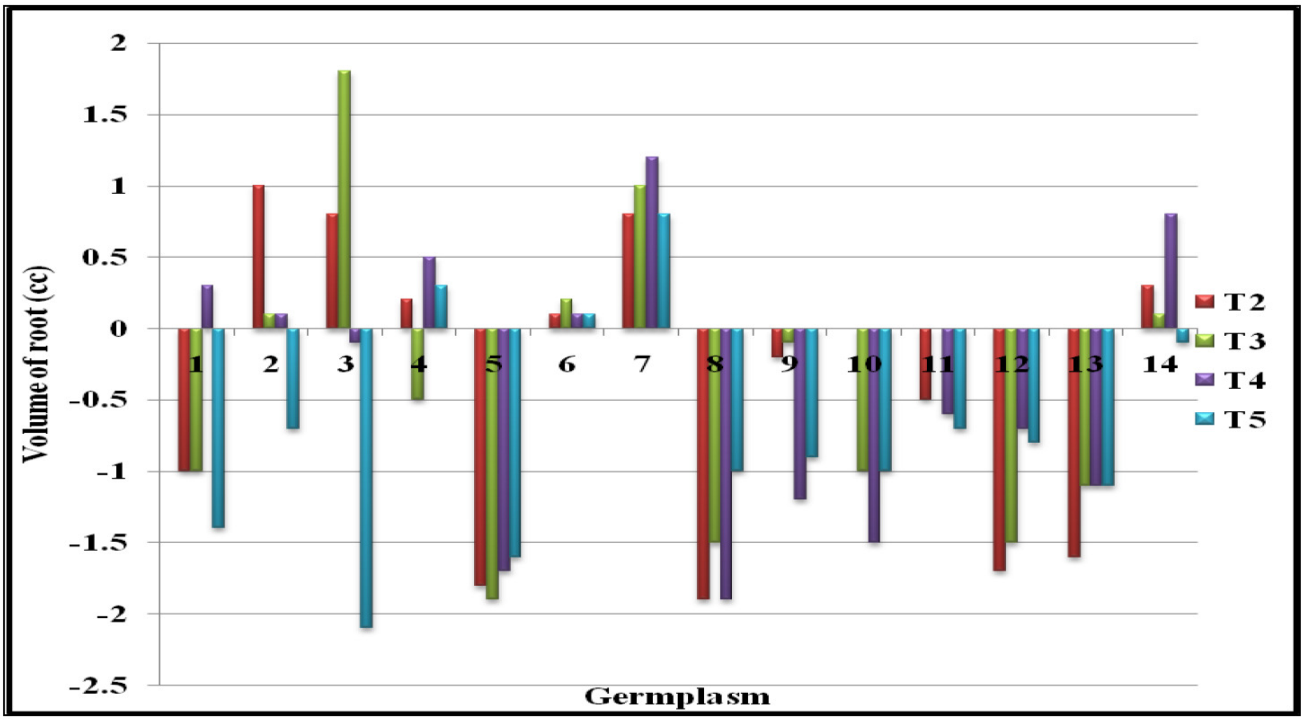

Fig 2: Reduction in root volume of fourteen citrus germplasm under various aluminium concentration

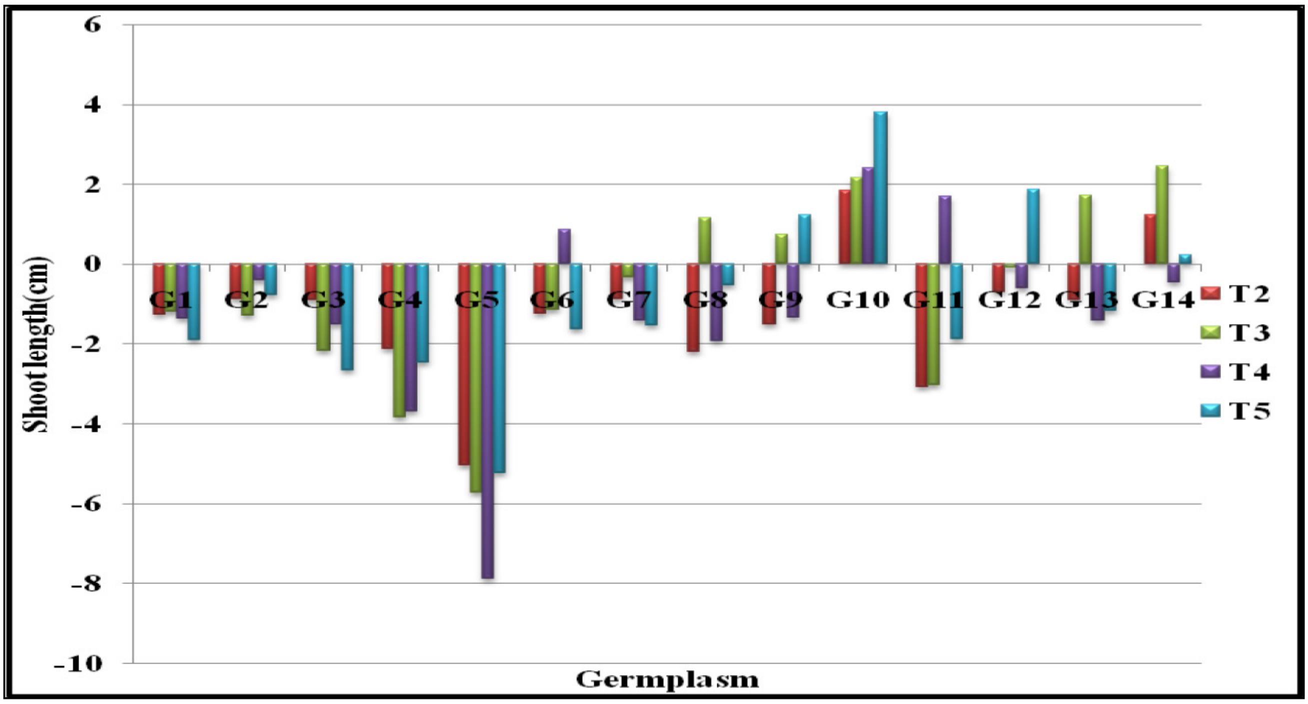

Fig 3: Reduction in shoot length of fourteen citrus germplasm under various aluminium concentration

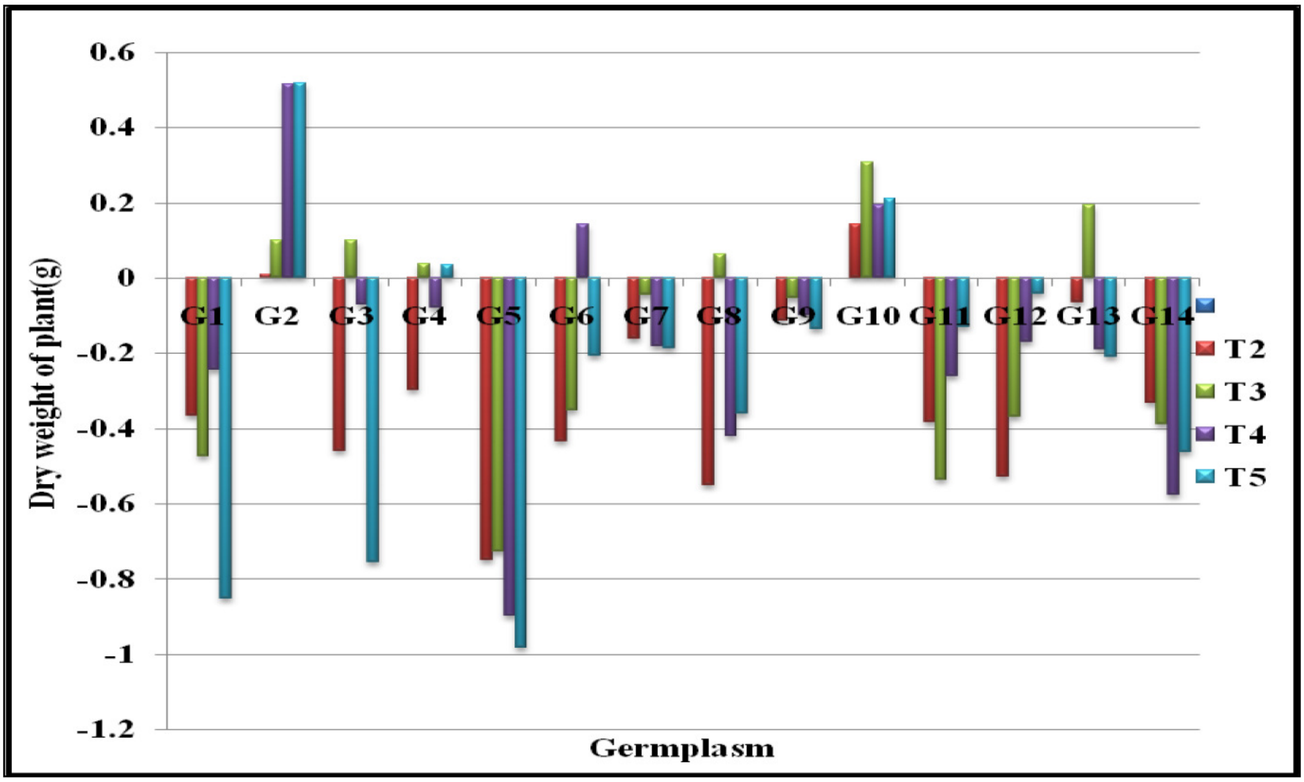

Fig 4: Reduction in dry weight of plant (DWP) of fourteen citrus germplasm under various aluminium concentration

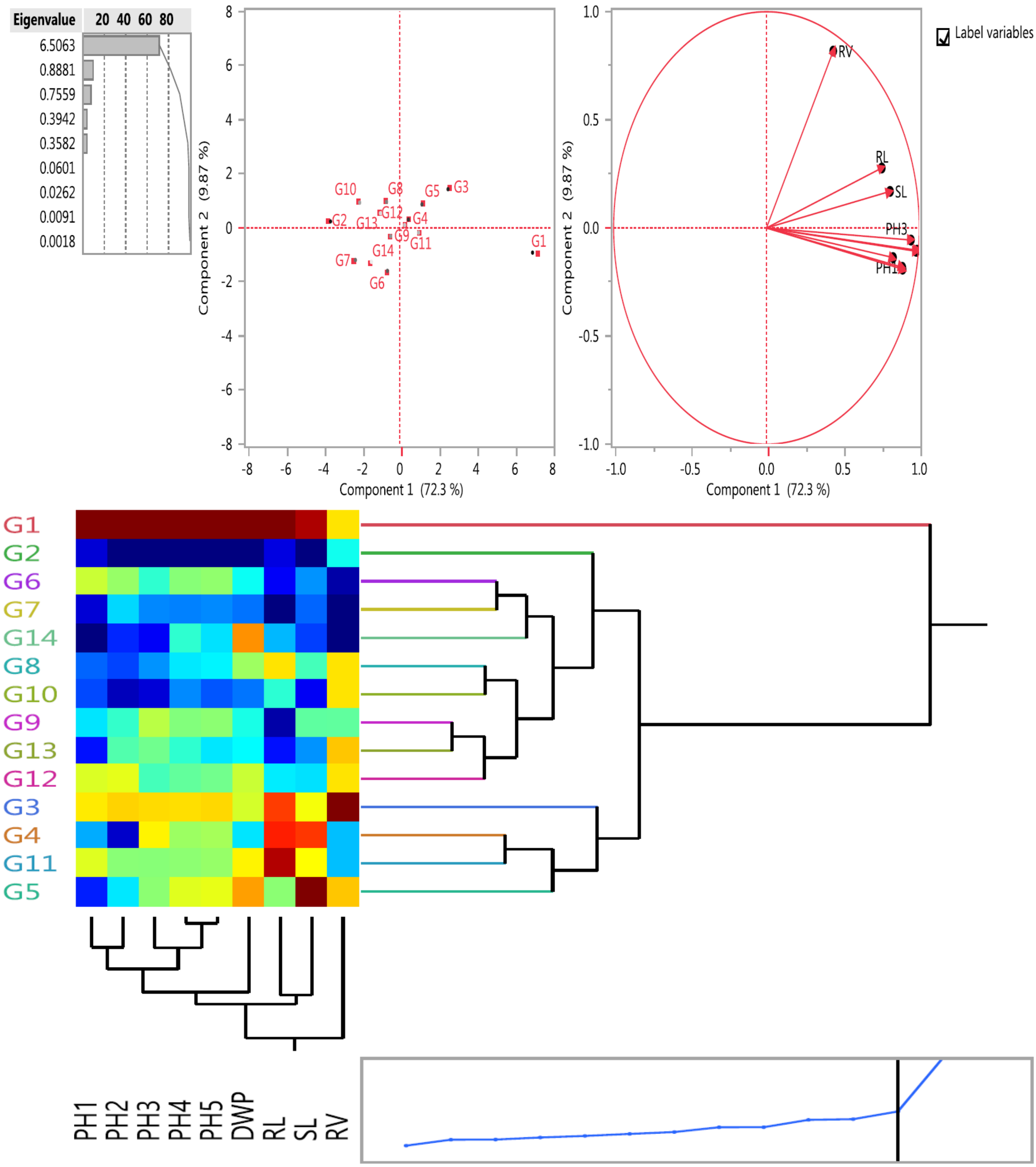

Fig 5: PCA and two way cluster analysis of citrus germplasm with plant parameter at A0

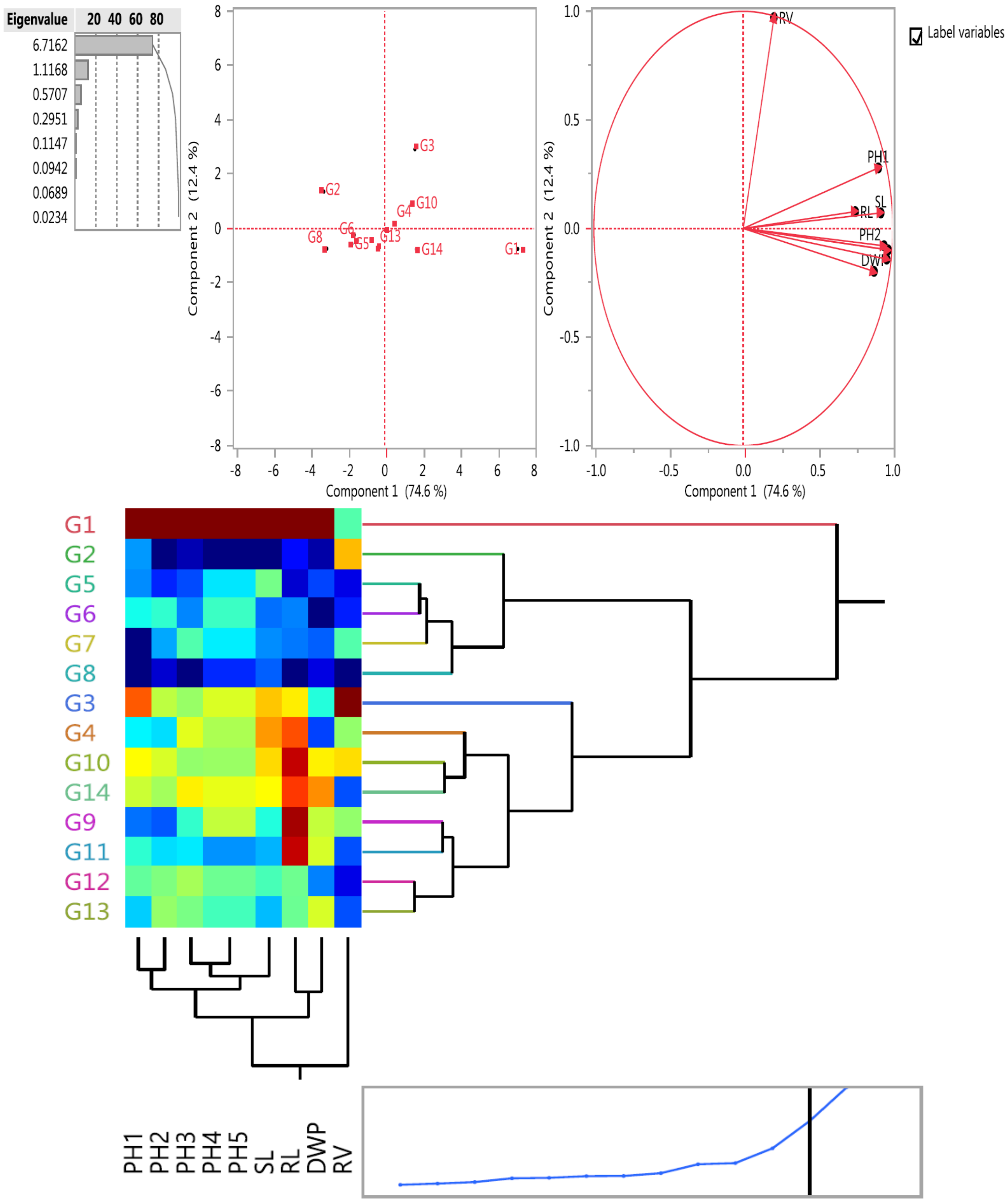

Fig 6: PCA and two way cluster analysis of citrus germplasm with plant parameter at A50

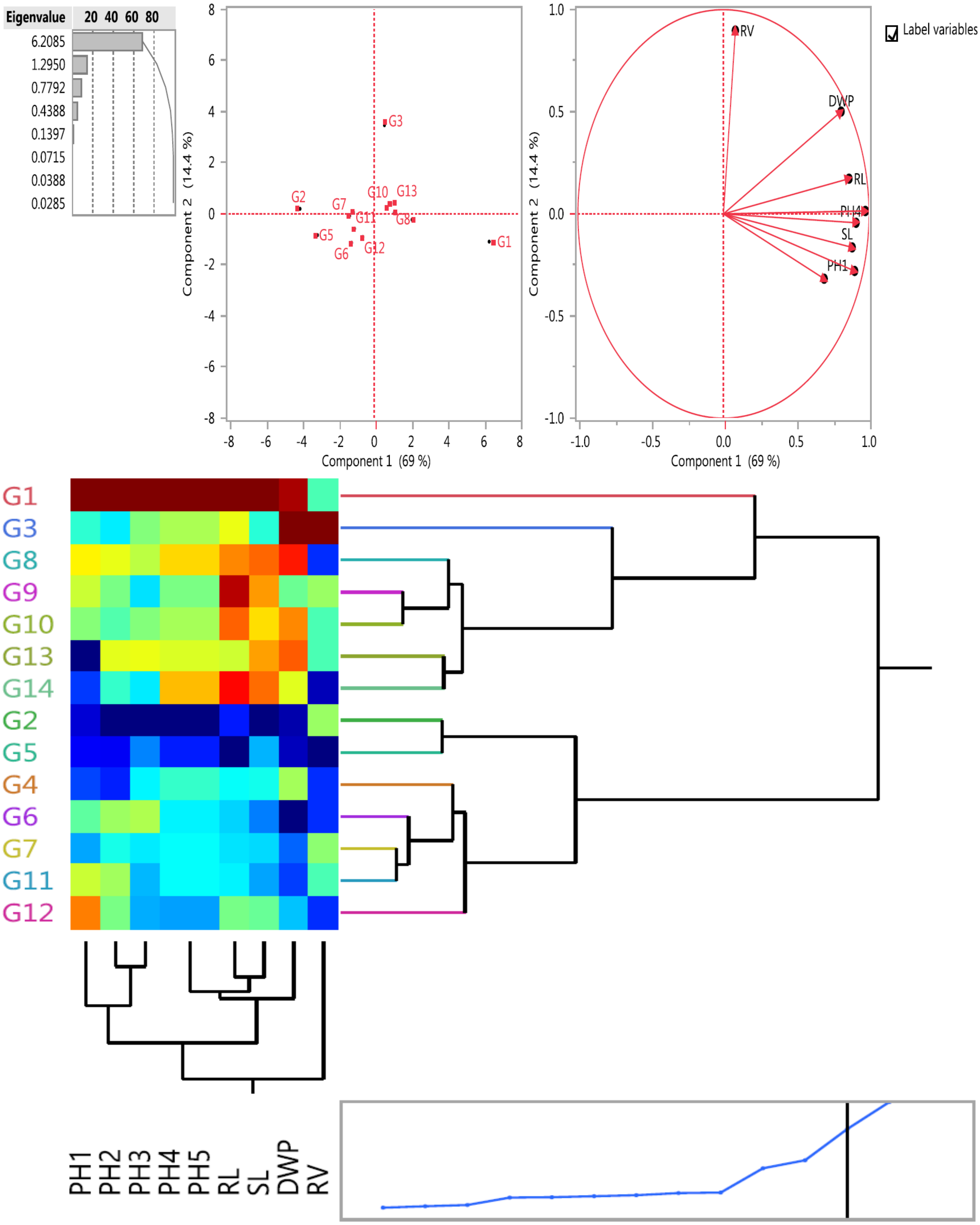

Fig 7: PCA and two way cluster analysis of citrus germplasm with plant parameter at A100

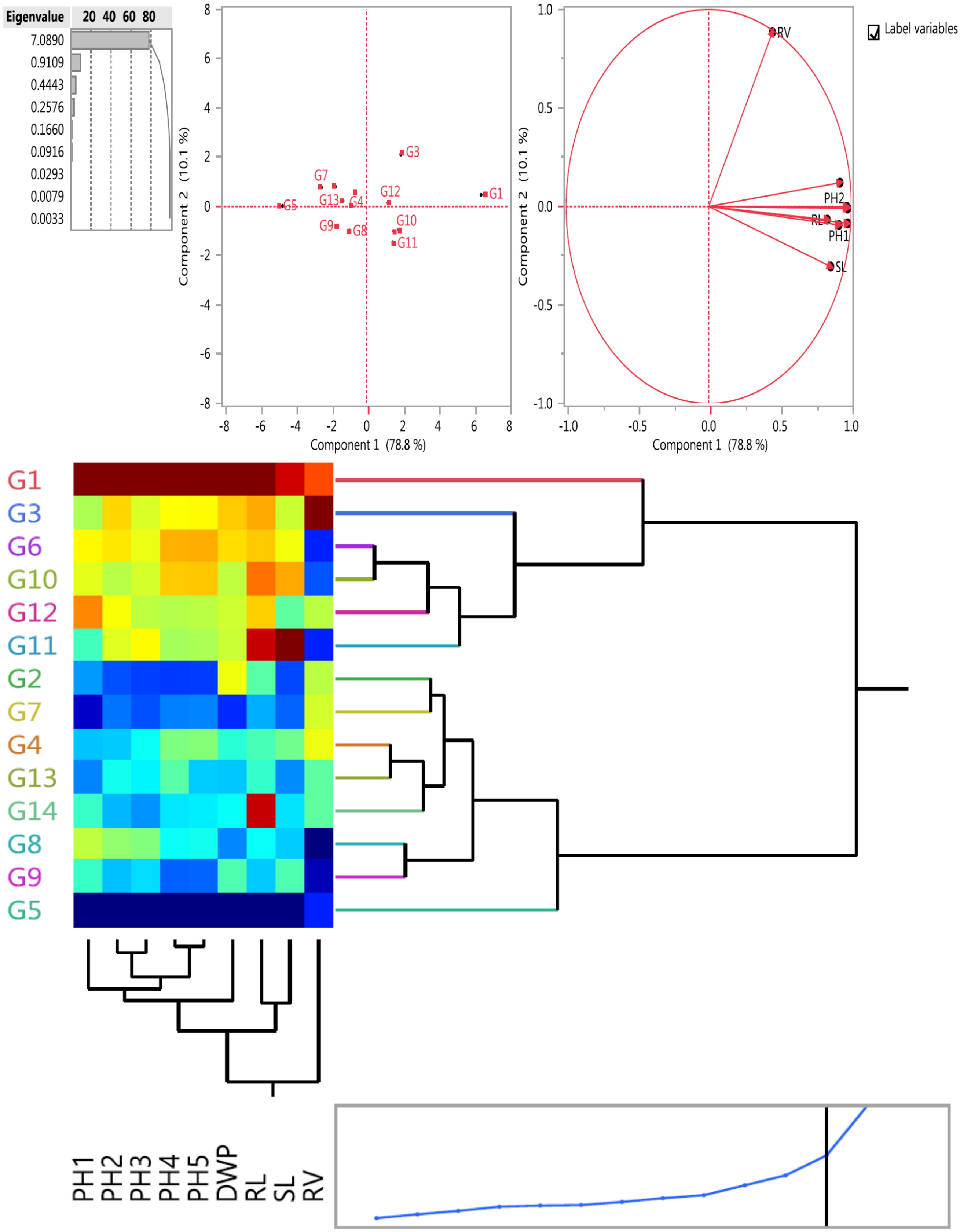

Fig 8: PCA and two way cluster analysis of citrus germplasm with plant parameter at A200

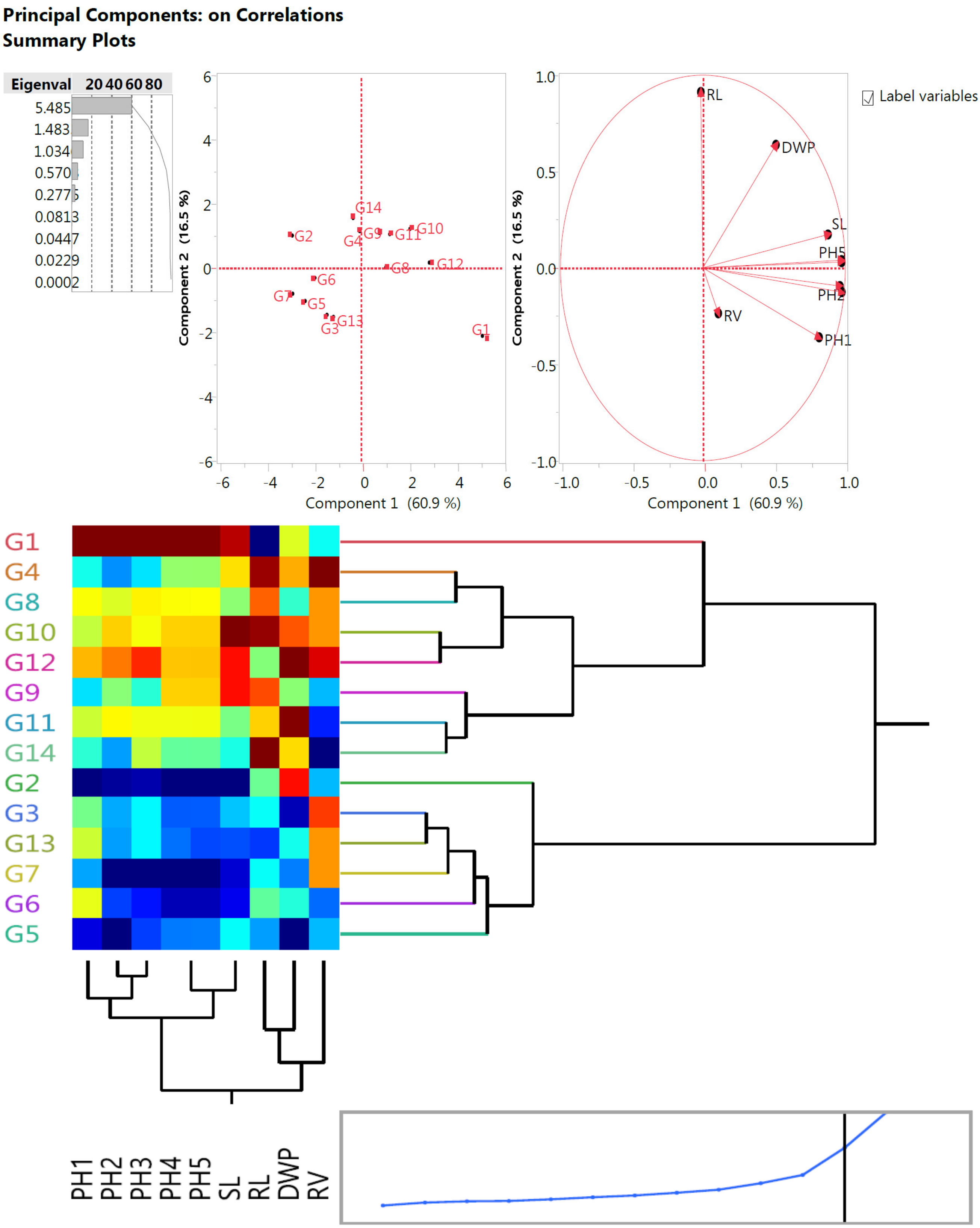

Fig 9: PCA and two way cluster analysis of citrus germplasm with plant parameter at A400

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