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TOTAL CALCIUM IN VARIOUS GROWTH STAGES OF BER *(ZIZIPHUS MAURITIANA LAMK)* GENOTYPES DIFFERING POWDERY MILDEW REACTIONS

P. NALLATHAMBI, C. UMAMAHESWARI and B.S. NATHAWAT
Plant Pathology Laboratory, Central Institute for Arid Horticulture (ICAR), Bikaner-334006, Rajasthan, India.
E-mail : ciah@hub.nic.in

Analysis of constitutive calcium ion in different growth stages of leaf and fruit samples ber genotypes revealed that tender leaves and fruits of susceptible types contain significantly less calcium percentage ranging from 0.45 to 0.55 in tender leaves; 0.53 to 0.68 in maturing leaves; 0.68 to 0.72 in matured leaves; 0.12 to 0.15 in tender fruits and 0.19 to 0.28 in matured fruits as compared to resistant genotypes. Out of 31 genotypes, maximum content of 0.99% calcium was estimated in matured leaves of wild species (*Z. nummularia)* followed by 0.95 in cv. Kathapal and Sanour-3 and 0.90% in Seo which are resistant to powdery mildew reactions under field conditions. Cumulative analysis of data revealed that tender leaves and fruits of ber genotypes are appropriate stage for getting consistent results. Constitutive level of calcium ion of ber and possible use for screening genotypes against powdery mildew reactions are discussed.

Keywords : Ber; Calcium; Germplasm; Powdery mildew; Resistance; *Ziziphus mauritiana.*

Introduction

Powdery mildew of ber *(Ziziphus mauritiana)* is a prime disease, causes intense reduction in productivity and quality of fruits and the filed screening on identification of resistant genotypes is resource intensive. Different biochemicals, such as calcium (Ca^{+2}) as major macronutrients are essential for the growth of cells and its effect on disease resistance. A study of healthy leaves from 5 resistant and 5 susceptible cultivars of pea against *Erysiphae polygoni* revealed quantitative variations in sulphur, phosphorus, calcium and magnesium and always higher in the resistant cultivars[1,2]. Calcium is required for activation of the resistant mechanisms and that of the ml-0 mutation affects calcium regulation in the cell, resulting in a elevated calcium ion level in the powdery mildew resistant isolines of barley[2]. Free cytoplasmic calcium has been postulated to play a role in preventing powdery mildew (*Erysiphe graminis* f.sp. *hordei)* in a series of homozygous mutants of barley[3]. Despite, attempts were not made to find out the quantitative variation in calcium ion in ber genotypes which are differing to powdery mildew reactions and therefore,presently, quantification of constitutive calcium in different stages of leaf and fruit samples of ber germplasm was carried out and the results are discussed.

Materials and Methods

Powdery mildew incidence intensity varies with growth stages of leaves and fruits of ber cultivars. Therefore, from each test genotypes which have been evaluated for powdery mildew reactions under field conditions[4]. Tender (immature), maturing and matured (old) leaves and immature or tender (pea size) and matured fruits were taken up for analysis of calcium by Flame photometrically as per the standard procedure described[5]. Samples were prepared from susceptible cultivars such as Gola, Umran, Seb and Mundia; and resistant cultivars viz., Rohtak Safeda, Darki-1, Chonchal, Govindgargh, Dandan, Narma, Sanour-1, Shamber, Badami, Safeda, Jhajjar Selection, Glory, Mirchia, KalaGola, BS-75-3-1, Narikela, Villaiti, Seedless, ZG-2, Saferchandi, Illaichi, Kathapal, Seo, Sanour-3, Kishmis, Chinese and *Z. nummularia* (wild) and analyzed repeatedly. Each sample was replicated 3 time in completely randomized block design. The critical level of ODD's ratio and range of confidential interval were worked out statistically to find out sampling of particular growth stage. Based on these two scale, calcium content in different growth stages of ber genotypes was compared and the results are discussed.

Results and Discussion

Powdery mildew is economically important disease in most of the commercial cultivars of ber and therefore, identification of resistant varieties is being carried out routinely. Being perennial fruit crop, field screening is resources intensive and alternative methods are warranted. With advent of biochemical attributes, rapid screening would not only minimize the resources but also possible for recurrent testing. Out of various biochemical indices, constitutive calcium in hosts plays critical role on resistance before or after the attack of plant pathogens. Presently, total calcium was analyzed in ber genotypes.

Table 1. Percent calcium in ber genotypes differ in reaction to powdery mildew.

Genotypes/ Growth stages	Calcium ion % in leaves and fruits of ber					
	TL	ML	OL	TF	MF	Reactions
Gola	0.46	0.54	0.68	0.12	0.19	S
Umran	0.47	0.68	0.69	0.14	0.20	S
Seb	0.50	0.66	0.67	0.13	0.28	S
Mundia	0.55	0.61	0.72	0.15	0.27	S
Rohtak Safeda	0.63	0.76	0.77	0.36	0.40	R
Darki-1	0.68	0.75	0.76	0.32	0.35	R
Chonchal	0.65	0.68	0.71	0.30	0.37	R
Govindgarh	0.57	0.61	0.62	0.28	0.30	R
Dandan	0.62	0.67	0.68	0.29	0.33	R
Narma	0.58	0.66	0.71	0.25	0.30	R
Sanour-1	0.56	0.57	0.63	0.17	0.18	R
Shamber	0.65	0.72	0.70	0.32	0.37	R
Badami	0.60	0.71	0.72	0.31	0.35	R
Safeda	0.57	0.61	0.68	0.32	0.38	R
Jhajjar Selecton	0.65	0.66	0.70	0.23	0.29	R
Glory	0.67	0.80	0.82	0.24	0.35	R
Mirchia	0.78	0.81	0.85	0.30	0.36	R
KalaGola	0.70	0.74	0.76	0.27	0.33	R
BS-75-3-1	0.61	0.66	0.76	0.22	0.32	R
Narikela	0.56	0.69	0.72	0.32	0.34	R
Villaiti	0.60	0.67	0.71	0.31	0.34	R
Seedless	0.57	0.60	0.61	0.32	0.33	R
ZG-2	0.73	0.75	0.80	0.30	0.31	R
Saferchandi	0.79	0.72	0.87	0.39	0.40	R
Illaichi	0.65	0.70	0.78	0.37	0.38	R
Kathapal	0.85	0.88	0.95	0.32	0.38	R
Seo	0.79	0.80	0.90	0.32	0.39	R
Sanour-3	0.85	0.89	0.95	0.20	0.35	R
Kishmis	0.66	0.71	0.82	0.28	0.32	R
Chinese	0.79	0.82	0.88	0.15	0.28	R
Z.numularia (wild)	0.80	0.83	0.99	0.38	0.45	R
C.D (p=0.05	0.0144	0.0149	0.0151	0.0474	0.0163	
ODD's ratio	53	53	6.625	424	NS	
Confidence Interval	4.7333 to 593.38	1.386 to 225.0	0.37 to 116.82	24.04 to 7476.77	NS	

TL : Tender Leaves, ML : Maturing Leaves, OL : Old Leaves, TF : Tender Fruits
MF : Matured Fruits, S : Susceptible, R : Resistant; Values are means of 4 replicates.

Out of 31 genotypes, less calcium percentage ranging from 0.46 to 0.55 in tender leaves; 0.54 to 0.68 in maturing leaves; 0.68 to 0.72 in matured leaves of susceptible cultivars was recorded (Table 1). The matured leaves recorded more Ca^{+2} content and maximum of 0.99% calcium was estimated from matured leaves of *Z. nummularia* followed by 0.95% in cvs. Kathapal, and Sanour-3 and 0.90% in cv. Seo, which showed resistance reactions under field conditions. Intermediate growth stage (maturing) and immature leaves of these genotypes were also enriched with high Ca^{+2} content. Fruits of wild species *(Z. nummularia)* contained high Ca^{+2} percentage than commercial cultivars. In commercial cultivars, calcium content was low in tender fruits than matured fruits. However, tender fruits of majority of the resistant genotypes contained high calcium (0.30 to 0.39%) as compared to 0.12 to 0.15% in tender fruits and

0.19 to 0.28% in matured fruits susceptible types. In general, ber fruits contain less calcium content followed by tender/ young leaves, which are highly susceptible for powdery mildew infection.

However, sampling of appropriate growth stages of leaves/fruits is essentially required to get consistency in results and comparison of resistance. Based on the ODD's ratio and Confidence interval (CI) analysis on identification of suitable growth stage, tender fruits are having high values being 24.04 to 7476.77 of CI and 424 of ODD's ratio followed 4.7333 of CI and 53 of ODD's ratio for tender leaves and therefore these two stages would be more appropriate for sampling and analysis of calcium content correlating this particular parameter with powdery mildew resistance. The over all results can be summaried as ber leaves are having high level of calcium than fruits. Accumulation of Ca^{+2} as one of the biochemical barriers act as systemic molecules as messenger on disease resistance and hence more concentration of this ion could be one of the biochemicals for ber powdery mildew resistance.

Four fungal diseases of ber viz., *Oidium ziziphi (O. erysiphoides* f.sp. *ziziphi*), *Phytophthora palmivora, Armillaria mellea* and *Cristulariella pyranidalis* are caused by deficiencies of certain minerals including calcium. In present investigation also, susceptibility of cv. Gola, Umran, Seb and Mundiya is perhaps, due to less Ca^{+2} ion present corroborating with the earlier report[6]. Low level of calcium in tender fruits of susceptible cultivars may also favour rapid infection of the pathogen which, is commonly observed and this fact is in agreement with the earlier findings[7] that the most susceptible fruits of banana had low calcium concentration. The Ethiopian barley cv. CI 3926/3 was more resistant to powdery mildew than Kenya but the level of dissease (*Erisyphe graminis* DC) resistance varied depending on the $Ca(NO_3)_2$: KH_2PO_4 ratio[8]. Incubation of partially dissected coleoptiles of a pair of ml-o resistance and susceptible barley lines in 10mM $Ca(NO_3)_2$ resulted as a marked expression of ml-o resistance depending on calcium[9]. Disease resistance in fruit can be enhanced by strengthening or stabilizing the cell walls with calcium so that they are resistant to diseases[10]. Calcium is implicated in certain fruits/pathogen interactions. In case of apple, calcium deficiency causes weakning and sensitization of the fruit to certain pathogenic agents[10,11]. In present investigation also tender fruits from resistant genotypes had high Ca^{+2} cncentrations as compared to susceptible cultivars.

Difference in concentrations of Ca^{+2} in various growth stages of leaves as well as fruits may be attributed with the biosynthesis and accumulation in accordance with the other physiological process of the host. Elicitation of other defence chemicals can be more effective in the presence of Ca^{+2} ion[3]. In some cases, the phytoalexin synthesis may also be stimulated by the calcium ions. Therefore, calcium may be considered as an essentially required element for the inhibition of powdery mildew pathogen. Perhaps, low level of Ca^{+2} in early growth stages of leaves and fruits may be constructive for the rapid infection and colonization of powdery mildew patogen under favorable conditions. Furthermore, based on the ODD's ratio and confidential interval analysis of present data, tender fruits could yield best result and therefore, to screen out large number of genotypes at early stage (nursery/seedling/budded plants), tender leaves would be appropriate and for field tender fruits could yield supportive results.

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