Ascorbic acid content, pest-pathogen preference and their phenological association in chilli (*Capsicum* spp)

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ABSTRACT

The landraces of *Capsicum* belonging to four major cultivated species, collected from various agro-ecological zones (AEZ) of Kerala were evaluated based on the ascorbic acid content and natural incidence of pests and diseases. Association of ascorbic acid was checked with 80 different characters comprising 40 qualitative and 32 quantitative characters, incidence of pest and diseases, species, horticultural type as well as AEZ differences. Association of bacterial wilt, dieback, mite, thrips and *Spodoptera* incidences with qualitative features was also evaluated. Species specific pigmentation and phenomics-based association patterns recorded in the present study will be helpful to sort out promising genotypes from a large pool of genetic resources and to fine tune selection criteria for ascorbic acid content and biotic resistance in chilli.

Keywords: Ascorbic acid; bacterial wilt; mite; thrips; dieback; *Spodoptera*

INTRODUCTION

Chilli (*Capsicum*) is an important solanaceous crop which finds use as vegetable, spice, medicine, self-defense sprays and animal repellent. Genus *Capsicum* comprises around 40 species out of which five viz *Capsicum annuum, C chinense, C frutescens, C baccatum* and *C pubescens* are cultivated. Cultivation of *C pubescens* is restricted to highlands of south America whereas the other four species are distributed all over the world (van Zonneveld et al 2015).

Genus *Capsicum* includes a large number of horticultural types as a result of introgressive hybridization, multiple domestication events, genome peculiarities, pollination adaptation and phenotypic plasticity (Smith and Heiser 1951, Qin et al 2014). Diversity is evident not only in the morphology of the fruit but also in its biochemical profile. A large number of biochemical constituents like capsaicinoids, ascorbic acid, carotenoids, flavanoids, xanthophyll, polyphenol, tannins (Giuffrida et al 2013, Garcia-Gonzalez and Silvar 2020, Gonzalez-Lopez et al 2021) etc are present in the chilli fruits which decide the colour, taste and pungency. Relative concentrations of different compounds make each landrace unique and determine its suitability to different niche markets and processing industry.

The production of chilli is hampered by many diseases and pests. Major diseases of chilli include bacterial wilt (*Ralstonia solanacearum*), anthracnose and dieback (*Collectotrichum* spp) and chilli leaf curl virus disease (begomoviruses) (Oo and Oh 2016, Chandrasekhar et al 2017, Thakur et al 2018). In summer months, yield and quality of produce is affected by sucking pest complex including white fly (*Bemisia tabaci*), green peach aphid (*Myzus persicae*), yellow mite (*Polyphagotarsonemus latus*), thrips (*Scirtothrips dorsalis*) and jassids (*Amrasca biguttula biguttula*) (Aarwe et al 2020). *Spodoptera* species damages leaves, tender shoots and fruits in chilli (Madala et al 2020). Biochemical profile and secondary metabolites in plants may interfere with growth, fecundity and reproduction of pests and pathogens leading to biochemical basis of resistance (Kehr 2006, Yang et al 2011, Chandrasekhar et al 2017).

Kerala, the southernmost state of India is broadly classified into three main agro-ecological zones (AEZs) viz western coastal plain (AEZ 1), midland (AEZ 2) and eastern hill zone (AEZ 3) based on altitude, geography, soil pattern, precipitation and ecological conditions (Rajasekharan et al 2015, Anon 2020). The present study was undertaken to evaluate the content of ascorbic acid in the landraces collected from Kerala to know the resistance level of these landraces against natural incidence of pests and diseases and to understand relationship of ascorbic acid as well as biotic stress incidence with phenological features. Ascorbic acid content and biotic stress tolerance were assessed in relation to species, horticultural types and AEZs also. Present study will be helpful in sorting out superior genotypes from large germplasm resources and in indirect selection of accessions with high ascorbic acid content and biotic stress tolerance using correlated, more reliable and easily identifiable phenological features.

MATERIAL and METHODS

One hundred and thirty landraces belonging to four cultivated species of *Capsicum* viz *Capsicum annuum, C chinense, C frutescens* and *C baccatum* formed the material for the present investigations. Except two from Mizoram, all the landraces were collected from various AEZs of Kerala. Genotypes were characterized at ICAR- National Bureau of Plant Genetic Resources, Regional Station, Thrissur, Kerala based on 40 qualitative characters, 32 quantitative characters and ascorbic acid content. Natural incidence of pests and diseases (bacterial wilt, dieback, mite, thrips and army worm) during the above period was also recorded as present or absent. Ascorbic acid content was estimated from fully ripened fruit as per the procedure given by Sadasivam and Manickam (1996).

Logarithmic transformation of ascorbic acid content was done to normalize the observations. Association of ascorbic acid content with qualitative characters was analysed through one way ANOVA. Based on ascorbic acid content, landraces were grouped into three classes viz low, medium and high using mean ± standard deviation and association of ascorbic acid levels with qualitative characters were revalidated using chi-square analysis. Phenotypic correlation between ascorbic acid content and quantitative characters was also estimated. Relationships of biotic stress incidence with different qualitative character states as well as species, horticultural type and AEZs were estimated using chi-square analysis. Statistical analysis was done using SPSS and GRAPES software (Gopinath et al 2021).

RESULTS and DISCUSSION

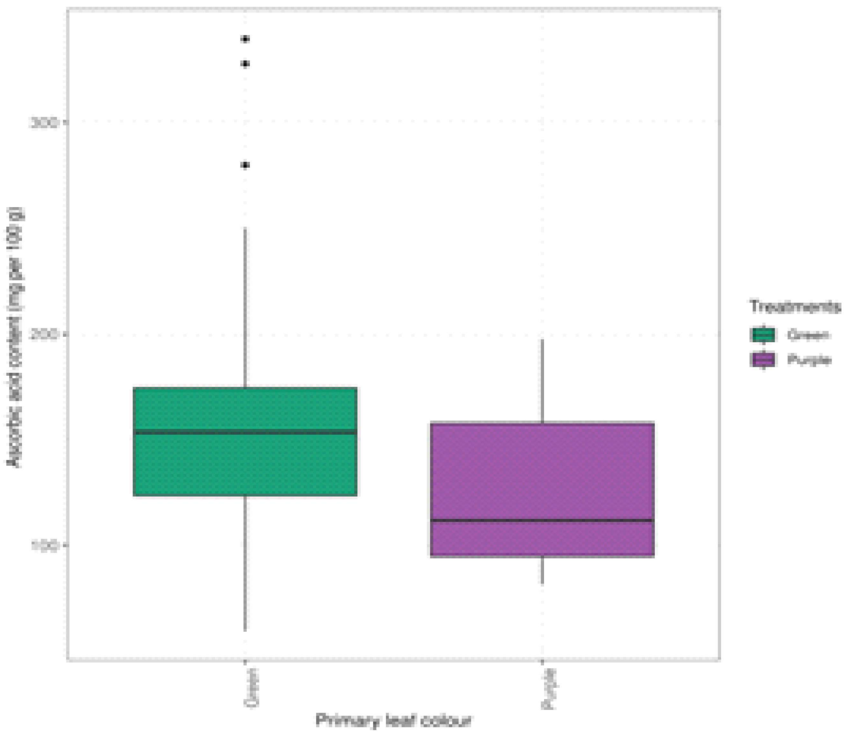
Ascorbic acid content and phenological association

Ascorbic acid content varied from 59.52 to 339.29 mg per 100 g of ripe fruit with a mean of 152.24 ± 4.22. Huge variability in ascorbic acid content is quite discernible as it is controlled by endogenous factors like genetic constitution (Chattopadhyay et al 2011, Litoriya et al 2014, Yatung et al 2014), feedback mechanism of genes (Alos et al 2013) as well as exogenous factors like drought (Kopta et al 2020), maturity, weather and post-harvest handling methods (Bhattacharya et al 2010). Landraces with green primary leaves, compact growth habit, non-pigmented calyx, green immature fruits and absence of petal spots were characterized with high ascorbic acid content (Table 1, Figs 1, 2). No significant variation was observed in ascorbic acid content with respect to difference in species, geographical origin as well as pest and disease incidence. The landraces with slow maturing nature as well as long fruits tended to have higher ascorbic acid levels (Table 2). Plant height, height to first bifurcation and stem diameter exhibited significant negative correlation with ascorbic acid content. Differentiation of ascorbic acid content with respect to fruit and leaf colouring pigments is in agreement with the work of Simonne et al (1997), Gadal et al (2003) and Patel et al (2015). Gonzalez-Lopez et al (2021) reported similarity of *C annuum* - *C frutescens* species as well as *C chinense* - *C baccatum* species in ascorbic acid with higher content in former group. They also observed significant differences in ascorbic acid content according to ecological-geographical origin of accessions. Kaur and Nayyar (2014) observed higher endogenous levels of ascorbic acid in stress-tolerant genotypes. The finding of positive association of ascorbic acid content and fruit length is in agreement with the work of Renthlei (1991), Manju and Sreelathakumary (2002) and Kumar

Table 1. Distribution of ascorbic acid and natural pest incidence among various descriptor states in chilli

Trait	Descriptor	Trait distribution in different descriptor states	
Ascorbic acid content of ripe fruit (mg/100 g)	Colour of primary leaf	Green (156.28)	Purple (123.43)
	Plant growth habit	Compact (153.12)	Erect (130.85)
	Calyx pigmentation	Non-pigmented (156.174)	Pigmented (131.84)
	Petal spot colour	Absent (156.01)	Present (137.85)
	Immature fruit colour	Green (160.92)	Purple (114.34)
Bacterial wilt incidence (%)	Species	*C chinense* (4.65)	*C frutescens* (25.93)
	Leaf colour	Purple (0)	Green (14.71)
	Nodal anthocyanin pigmentation	Present (2.33)	Absent (16.09)
	Fruit surface	Wrinkled (0)	Semi-wrinkled (21.21)
Mite incidence	Colour of primary leaf	Purple (0)	Green (22.81)
	Filament colour	Purple (8.33)	Yellow (26.00)
	Immature fruit colour	Purple (0)	White (38.89)
Thrips incidence	Flower position at anthesis	Erect (4.92)	Pendent (23.81)
	Immature fruit position	Erect (6.00)	Pendent (32.00)
	Fruit blossom end appendages	Absent (14.78)	Present (40.00)
	Persistence of fruit pedicel on stem	Strong (0)	Weak (38.46)
Dieback incidence	Species	*C annuum* (21.43)	*C frutescens* (74.07)
	Horticultural type	Ancho (10.00)	Tabasco (74.19)
	Branching habit	Sparse (19.05)	Dense (61.54)
	Stem colour	Green with purple stripes (0)	Green (43.02)
	Stem pubescence	Dense (19.23)	Sparse (48.72)
	Nodal anthocyanin pigmentation	Present (13.95)	Absent (45.98)
	Leaf density	Low (16.67)	High (53.33)
	Leaf hair density	Intermediate (12.5)	Dense (48.57)
	Flower position at anthesis	Pendent (28.57)	Erect (54.10)
	Corolla colour	White or purple (19.00)	Greenish yellow (48.61)
	Immature fruit colour	Green or purple (28.57)	White (77.78)
	Immature fruit position	Pendent (20.00)	Erect (60.00)
	Fruit shape at pedicel attachment	Truncate (18.42)	Acute (64.87)
	Fruit surface	Wrinkled (15.79)	Semi-wrinkled (50.00)
	Persistence of fruit pedicel on stem	Slight (17.95)	Intermediate (44.74)
	Persistence of ripe fruit to pedicel	Intermediate (23.91)	Strong persistence (41.67)
Spodoptera incidence	Species	*C chinense* (2.33)	*C annuum* (35.71)
	Horticultural type	Ancho and Cherry (0)	Anaheim (54.55)
	Hypocotyl colour	Purple (8.33)	Green (32.88)
	Leaf shape	Deltoid (0)	Lanceolate (34.62)
	Number of flowers/axil	Two or more (10.71)	One (35.19)
	Calyx margin	Entire (16.67)	Dendate (56.25)
	Corolla colour	Greenish yellow (15.28)	White (43.24)
	Anther lobe colour	Yellow (5.56)	Blue (40.54)
	Filament colour	Green (0)	White (39.13)
	Fruit shape	Round (8.70)	Elongate (40.98)
	Fruit shape at blossom end	Non-pointed (9.68)	Pointed (28.28)
	Fruit cross-sectional corrugation	Corrugated (0)	Slightly corrugated (35.00)
	Fruit surface	Wrinkled (5.26)	Smooth (37.78)
	Blossom end appendage	Present (0)	Absent (26.96)
	Annular constriction at pedicel end	Present (6.52)	Absent (33.33)
	Calyx margin of fruit	Entire (12.12)	Intermediate (35.94)
	Persistence of fruit pedicel on stem	Slight (7.69)	Strong persistence (40.00)
	Seed colour	Brown (5.56)	Yellow (26.79)

Only those descriptors that expressed significant association with ascorbic acid or pest incidence listed; Values in brackets are mean content of ascorbic acid or percentage incidence of a pest across a particular descriptor state



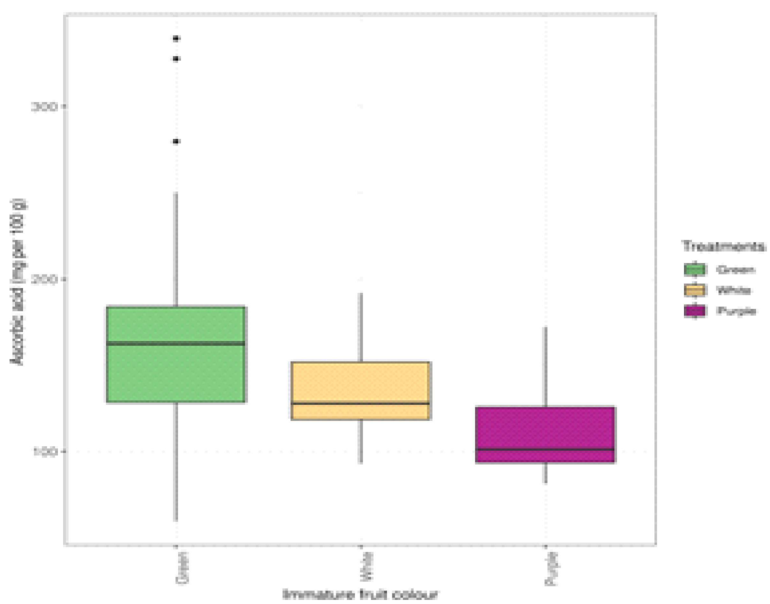


Fig 1 and 2. Box-plot elucidating association of ascorbic acid with primary leaf colour and immature fruit colour; twenty fifth percentile, median, seventy fifth percentile, range and outliers presented

et al (2003). However previous studies reported positive (Manju and Sreelathakumary 2002) as well as negative association (Sharma et al 2010) of ascorbic acid with plant height.

Phenological association of pest and disease preference

The landraces with purple leaves, nodal anthocyanin pigmentation and wrinkled fruit surface were less prone to bacterial wilt infection (Table 1). None of the landraces with purple leaves had bacterial wilt symptoms. Similarly landraces with wrinkled fruit surface were totally devoid of wilt symptom. Landraces with purple pigmentation were characterized with less incidence of mite infestation (Fig 3). Mite infestation had significant negative correlation with purple primary leaf, purple coloured filament and purple fruit colour at immature stage. Previous studies indicated influence

Table 2. Correlation revealing association of ascorbic acid content with quantitative characters

Character	Ascorbic acid content
Length of primary leaf (mm)	0.132
Width of primary leaf (mm)	0.086
Plant height (cm)	-.223*
Plant canopy width (cm)	-0.089
Stem length from ground level to first branch (cm)	0.082
Height to first bifurcation (cm)	-0.271**
Stem diameter (mm)	-0.299**
Length of mature leaf (cm)	-0.094
Width of mature leaf (cm)	-0.053
Leaf base angle	-0.161
Leaf tip angle	0.031
Days to flowering	-0.068
Petal length (cm)	0.072
Length of anther lobe (cm)	0.088
Filament length (cm)	0.079
Days to fruiting	0.027
Fruit set (%)	0.069
Fruit bearing period (days)	-0.035
Days to maturity of fruit	.227**
Fruit length (cm)	.173*
Fruit width (cm)	0.007
Fruit pedicel length (cm)	-0.128
Fruit weight (g)	0.091
Fruit wall thickness (mm)	-0.002
Number of locules	-0.103
Seed diameter (mm)	-0.063
1000-seed weight (g)	0.02
Number of seeds per fruit	0.153
Dry weight per fruit	-0.006
Fruit dry matter content (% DW)	-0.027
Number of fruits per plant	0.016
Yield (g)	0.054

of lot of biochemical features like tannins, phenols, chlorophyll, total sugars, protein content and mineral content in controlling mite incidence (Ahmed et al 2000, Rameash et al 2017, Latha and Hanumanthraya 2018). Landraces with purple pigmentation may be characterized with biochemical features that are less favourable to mite infestation. Incidence of thrips exhibited significant negative association with erect position of flowers, absence of fruit blossom end appendage, erect position of fruits and strong persistence of fruit pedicel with stem (Fig 4). Erect flower, erect fruit position and strong persistence of fruit pedicel on the stem are considered as wild features in chilli (Carvalho et al 2014). Hence it could be inferred that landraces retaining wild characters had deterrence to thrips infestation whereas those with more of domesticated characters were more prone to infestation.

Dieback incidence was found to be significantly low in landraces with sparse branching habit, green stem with purple stripes, dense stem pubescence, nodal anthocyanin pigmentation, sparse leaf pubescence, low leaf density, non-erect position of flower and fruit pedicel, white or purple corolla, non-white (green or purple) immature fruit colour, wrinkled fruit surface, low persistence of ripe fruit pedicel on the stem, low persistence of ripe fruit on the pedicel and truncate fruit shape at pedicel attachment. Present study revealed high vulnerability of *C fruescens* genotypes to dieback disease. Association of secondary features of *C frutescens* viz erect flower and fruit position, greenish yellow corolla, white immature fruit colour and semi-wrinkled fruit surface (Greenleaf 1986, Eshbaugh 2012) with dieback incidence may be due to high vulnerability of that species to dieback.

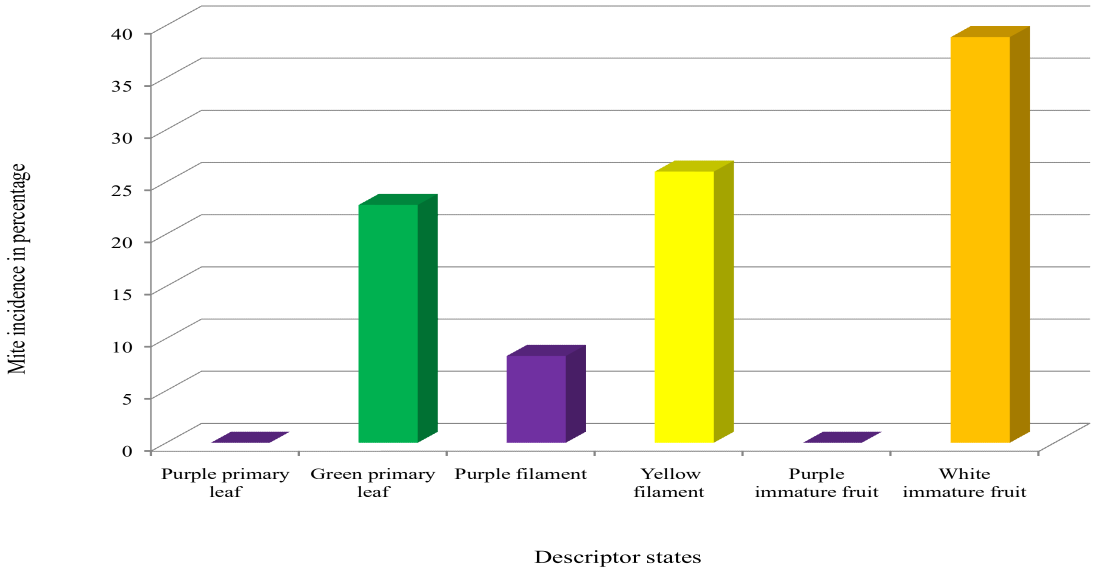


Fig 3. Natural incidence of mite across contrasting descriptor states in chilli

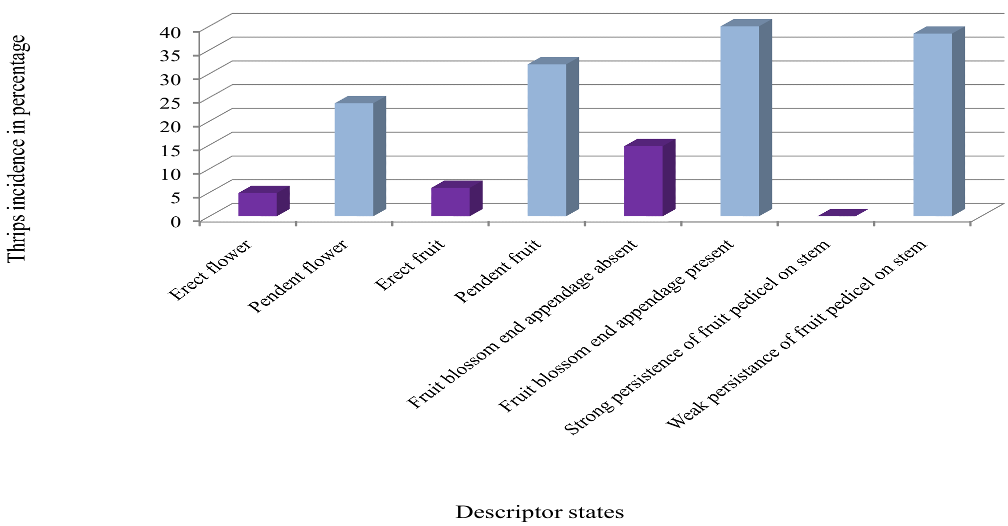


Fig 4. Natural incidence of thrips across contrasting descriptor states in chilli

Spodoptera infestation was significantly lower in landraces with purple hypocotyl, deltoid or ovate leaf shape, entire calyx margin, greenish yellow corolla, yellow anther lobe, green or yellow filaments, non-elongate shaped fruits, non-pointed blossom end of fruit, fruit blossom end appendage, highly corrugated fruit cross section, wrinkled fruit surface, annular constriction at junction of pedicel and calyx, low persistence of ripe fruit pedicel on stem, brown seed colour and presence of more than two flowers per axil.

The present study revealed the low preference of *Spodoptera* towards *C chinense* types. Annular constriction is a key character of *C chinense* (Smith and Heiser 1957). Further, most of the cultivated types of *C chinense* are characterized by the presence of ovate leaves, entire calyx margin, highly corrugated fruits, wrinkled fruit surface and more than two flowers per axil. Negative association of these characters with *Spodopera* infestation may be due to deterrence of the pest to *C chinense*.

Table 3. Characters helpful for selection of chilli genotypes with high ascorbic acid content and low preference to pests and diseases

High ascorbic acid content	Low incidence of bacterial wilt	Low incidence of mite	Low incidence of thrips	Low incidence of dieback	Low incidence of *Spodoptera*
Green primary leaves, compact growth habit with less height, non-pigmented calyx, green immature fruits, absence of petal spots, slow maturing fruits, long fruits, speedy bifurcation of stem	Purple leaves, anthocyanin pigmentation on node, wrinkled fruit surface	Purple primary leaves, purple filament, purple immature fruit colour	Erect flowers and fruits, absence of fruit blossom end appendages, strong persistence of fruit pedicel to stem	Sparse branching habit, green stem with purple stripes, dense stem pubescence, anthocyanin pigmentation on node, low leaf density, pendent flower and fruit position, white or purple corolla, white anther lobe, green or purple immature fruit colour, truncate shape at fruit base, wrinkled fruit surface, slight persistence of fruit pedicel to stem	Purple hypocotyl colour, entire calyx margin, greenish yellow corolla, yellow anther lobe, green filament, wrinkled fruit surface with corrugated fruit cross section, round fruit shape, presence of fruit blossom end appendage, slight persistence of fruit pedicel to stem, brown seed colour

Among the cultivated species of the annuum complex, incidence of bacterial wilt and dieback was the highest in *C frutescens*. Bacterial wilt incidence and *Spodoptera* infestation were found to be the lowest in *C chinense*. *C annuum* landraces were characterized with lowest incidence of dieback symptoms and highest incidence of *Spodoptera* infestation. The report of absence of species specificity in bacterial wilt resistance through artificial inoculation study (Anusree 2020) is contrary to the results of the present investigations. In the present study, Ancho, Cayenne, Cuban and Wax groups were almost free from dieback incidence whereas Tabasco types were highly prone to disease. Landraces belonging to Ancho and Cherry groups were completely free from *Spodoptera* infestation.

Based on the phenological associations elucidated from the present study, correlated characters helpful for selection of genotypes with higher ascorbic acid and biotic tolerance were worked out (Table 3). This will be helpful for easy identification of desirable genotypes from a large group of germplasm. It will also enhance the accuracy of the selection programme during the field screening. Estimation of ascorbic acid is laborious, time consuming and one has to wait till fruit maturity. Further screening for pests and diseases needs specific climatic and environmental conditions. Under these circumstances, correlated characters which are less influenced by environment and easily identifiable at the seedling or vegetative stage will be advantageous.

CONCLUSION

The landraces used in the present investigations were significantly different in ascorbic acid content and preference to pests and diseases. Characters significantly associated with higher level of ascorbic acid content and low incidence of pests and diseases could help the breeder in the early selection of genotypes from large germplasm resources as well as to speed up genetic gain.

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