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Variability in morpho-physiological traits and antioxidant potential of kiwifruit (*Actinidia chinensis* Planch) in Central Himalayan Region

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Abstract

Due to reeling impact of climate change in the Central Himalayan Region, temperate fruits requiring high chilling are shifting towards higher elevations. Owing to the demand, exotic kiwifruit is taking the place of temperate fruit crops in hills. Hence, it is important to properly evaluate different traits for promoting cultivation of kiwifruit in the hills. In the present investigation, variability in morphological characters of five female varieties (Abbott, Allison, Bruno, Hayward and Monty) and one male variety (Tomuri) of kiwifruit was studied. Leaf length, leaf width, petiole length, petal length, petal width and flower diameter ranged from 12.83 – 16.43 cm; 12.33 – 16.47 cm; 7.23 – 13.43 cm; 2.13 – 3.03 cm; 1.63 –2.24 cm; 4.77–6.51 cm respectively. Moreover, these genotypes were evaluated for fruiting traits *viz.*, days to bud burst, days to end bud burst, fruit length, fruit width, fruits weight, fruit volume and days to harvesting which ranged from 115 – 127; 126 – 131; 5.70–7.57 cm; 3.50–4.73 cm; 53.10–69.17g; 66.50–88.30 cc, 285–322 respectively. Besides, ripe fruits were analysed for pH of juice, total soluble solids (TSS), total phenols, flavonoids, ferric reducing antioxidant power, DPPH assay and Vitamin C content which ranged from 3.6–4.9, 11.85–18.05 ºBrix, 106.18 – 156.18 mg kg^{-1} f.wt., 20.08–31.47 mg kg^{-1} f.wt., 82.41–137.19 mg of ascorbic acid equivalent antioxidant assay, 176.32–274.97 mg of ascorbic acid equivalent antioxidant assay, 41.3 – 83.6 mg $100g^{-1}$ f.wt., respectively. Substantial genotypic variability available in different varieties may be harnessed for cultivation and crop improvement.

Key words

Antioxidant, Kiwifruit, Vitamin C, Yield

Introduction

Kiwifruit belongs to the family Actinidiaceae, genus *Actinidia*. Genus *Actinidia* has more than 60 species out of these, some species have remarkable economic importance such as *A. chinensis* (Rassam and William, 2005). Kiwifruit (*Actinidia chinensis* Planch) is an economically and nutritionally important fruit crop which originated in China. It has gained worldwide popularity in recent years due to its wider climatic adaptability, delicious fruits with unique blend of taste, precocity as well as high nutritive and medicinal values (Zenginbal *et al*., 2007). In India kiwi fruit is comparatively a new crop which was introduced during the 1970s. Himalayan region harbours rich biodiversity and is one of the most vulnerable mountain ecosystems to climate change (Xu *et al*., 2009; Bawa *et al*., 2010) but there is paucity of systematic analysis of climate change and its impacts on agri-horticultural crops and local peoples livelihood (Shrestha *et al*., 2012).

Plant breeders require genetic variation (genotypes) for crop improvement. Response of a crop to environmental conditions varies from variety to variety. Environmental conditions during plant growth and development affect various morpho-physiological traits, flowering, fruiting behaviour and productivity. Fruit size, its quality and storability are affected by factors during growth and development. Warmer weather conditions enhance pigment development, stimulate fruit maturity and enhance skin colouration. But in temperate fruit production, low temperature requirement (chilling) is a limiting factor in several areas including the Central Himalayan Region. The amount of cold needed by a plant to resume normal spring growth following the winter period is commonly referred to as its "chilling requirement". Himalayan

Region, at present, is facing the challenges created due to increasing aridity, warmer winter season, variability in precipitation and unexpected frosts and storms (Renton, 2009) which largely affect the entire range of biodiversity, including agricultural and horticultural crops (Kala, 2013). In view of the impact of climate change on horticultural crops, there has been an increase in interest for new crops. The need for adaptation to climate change is an emerging focus of international policy (IPCC, 2007). Himalayan Region is more vulnerable to climate change as compared to other areas. Impact of climate change is more in perennial crops as compared to annuals. Therefore, two pronged strategy ought to be adopted *i.e.*, characterization and evaluation of available genetic resources as well as exploring options of new suitable crops.

Kiwifruit contains antioxidants, vitamins and phytochemicals called flavonoids that may have the power to neutralise unstable molecules called 'free radicals'. Free radicals are thought to be linked to chronic diseases, cancer and aging. Kiwifruit consumption has effects similar to the daily dosage of aspirin, often recommended by physicians to improve health of heart (Rush *et al.,* 2002). In addition, kiwifruit has laxative effects also (Rush *et al.,* 2002). Ascorbic acid (Vitamin C) in fruits is considered as an important component for human nutrition. More than 90% of the ascorbic acid in human diet comes from fruits and vegetables (Lee and Kader, 2000). Kiwifruit is a rich source of ascorbic acid also. Moreover, polyphenolic compounds (flavonoids) also have antioxidant characteristics and can account for some benefits associated with consumption of fruits and vegetables (Wong *et al*., 2006). Fruits contain numerous compounds which display antioxidant activities such as vitamin C, phenols etc. (Kondo *et al*., 2005). Commercial kiwifruit growing areas have expanded since 1990s. By 2010, the global kiwifruit planting area had reached over 150,000 ha. China (70,000 ha), Italy (27,000 ha), New Zealand (14,000 ha) and Chile (14,000 ha) account for about 83% of the world kiwifruit plantings, and global kiwifruit production represents about 0.22% of the total production for major fruit crops, with the majority of kiwifruit consumed as fresh fruit. Hence, the present study was carried out with an objective to evaluate genetic variability in morpho-physiological traits of kiwifruit varieties in the Central Himalayan Region and quantify antioxidants for harnessing the available potential.

Materials and Methods

A total of 6 exotic varieties (five female viz., Abbott, Allison, Bruno, Hayward and Monty and one male i.e., Tomuri) planted in 1993 were evaluated for three consecutive years (2010 – 2012) at an experimental site located at 1480 m asl altitude. Average annual precipitation of experimental site over the last ten years was 1200 mm -1800 mm. Terminal leaf length, terminal leaf width, terminal petiole length, petal length, petal width, flower diameter, fruit length, fruit width was measured in centimetres. Number of days from the Julian Day (1st January) to 5% bud burst was counted and expressed as days to bud burst. Similarly, the number of days from Julian Day to 95% bud burst was counted and expressed as days to end bud burst. Weight of ten representative fruits of different sizes was recorded with the help of electronic pan balance and the average was expressed as fruit weight (g). Volume of fruits was measured by dipping the fruits in measuring cylinder and measuring the volume of water replaced by fruits. Fully ripe fruits were used to measure the pH of juice by pH meter. Total soluble solids at full ripe stage (ready to eat) was measured with the help of refractometer and expressed as °Brix.

Total phenol content was estimated by spectro-photometric method using Folin Ciocalteu Reagent (FCR) method (Slinkered and Singleton, 1977). Total flavonoids were estimated by the method of Mărghitas *et al*. (2007). Ferric Reducing Antioxidant Potential (FRAP) assay has been done by measuring the change in absorbance at 593 nm due to reduction of ferric tripyridyltriazine (Fe^{III}- TPTZ) complex to the ferrous form as described by Benzie and Strain (Benzie and Strain, 1996). DPPH method of Brand-Williams *et al.* (1995) was used with modification. To determine reaction kinetics for kiwifruit extracts, reactions were initially monitored over a 24 hr period with readings recorded every 30 min for the first 2 hr, every 2 hr for the next 10 hr and every 6 hr thereafter. Most of the samples tested showed residual reactivity even after 24 hr. However, after 8 hr change in activity was very minimal for most of the samples. Hence, 8 hr was used as standard reaction time. $DPPH^{\cdot}$ was dissolved in methanol and kept at -20 ºC in dark prior to use. Sample extracts (150 µL) were reacted with 2850 µl of DPPH solution for 8 hr while shaking. Trolox was used as standard. Ascorbic acid estimation was done by spectrophotometric method using dichlorophenol indophenol dye solution (Davis and Masten, 1991).

Experiments were conducted in five replicates for three consecutive years. Three plants of each variety were maintained in each replication. Data presented are mean of the data recorded for three consecutive years from the same plant. Data for each parameter was evaluated for statistical significance using two way ANOVA to compare means considering variety and parameters as independent variables. Individual parameter between two varieties were assessed by computation of least significant difference taking 't' values for error degree of freedom at 5% level of significance.

Results and Discussion

Cultivation of fruit crop in an area depends on the availability of congenial climate and suitable variety. Climate can not be controlled in large scale cultivation but suitable variety may be selected for successful cultivation. In real sense, farmers are ground level breeders who select the best suitable variety for cultivation at large scale. Although, apparently there may be minor variability in plants of different varieties but variability in different

Table 1 : Variability in morphological characters of kiwifruit (*Actinidia chinensis* Planch)

Name of variety	Accession numbers	Leaf length (cm)	Leaf width (cm)	Petiole length (cm)	Petal length (cm)	Petal width (cm)	Flower diameter (cm)
Abbott (♀)	EC64094	13.60±0.65	15.37±0.73*	11.70±0.51	2.70±0.11	1.93±0.084	5.67±0.23
Allison (♀)	EC24672	13.07±0.64	12.57±0.62	7.3±0.31	2.90±0.13	1.63±0.078	5.90±0.27
Bruno (♀)	EC64090	14.20±0.71	13.77±0.62	10.70±0.48	2.57±0.12	2.23±0.098	5.47±0.24
Hayward (♀)	EC64093	14.50±0.72*	13.53±0.58	7.23±0.33	3.00±0.14	2.20±0.099*	6.30±0.29*
Monty (♀)	EC137263	12.83±0.60	12.33±0.56	13.43±0.64*	3.03±0.14*	2.24±0.097*	6.51±0.31*
Tomuri (♂)	EC264092	16.43±0.74*	16.47±0.77*	9.57±0.47	2.13±0.10	1.67±0.076	4.77±0.19

Values given in the table are mean ± SE; * Significant at $p \leq 0.05$

Table 2 : Variability in fruiting traits of kiwifruit (*Actinidia chinensis* Planch)

Name of variety	Accession numbers	Days to bud burst	Date to end bud burst	Fruit length (cm)	Fruit width (cm)	Fruits weight (gm)	Fruit Volume (cc)	Days to harvesting
Abbott (♀)	EC64094	124.0±5.31*	126.3±5.33	6.13±0.32	3.90±0.16	57.50±2.72	66.50±3.26	297±7.33
Allison (♀)	EC24672	120.0±5.42	127.0±5.27	7.57±0.35*	3.83±0.14	53.33±2.31	79.66±3.47	285±6.66
Bruno (♀)	EC64090	122.0±5.15	128.0±5.14*	5.97±0.24	3.80±0.14	53.10±2.21	72.50±3.34	308±7.50
Hayward (♀)	EC64093	127.0±5.23*	131.0±5.85*	6.17±0.31	4.73±0.19*	69.17±3.34*	88.30±4.02*	322±6.36*
Monty (♀)	EC137263	115.0±4.97	126.0±5.96	5.70±0.22	3.50±0.13	62.17±3.17	74.50±3.21	312±6.12
Tomuri (♂)	EC264092	119.0±5.08	128.0±5.42	—	—	—	—	—

Values given in the table are mean ± SE; * Significant at $p \leq 0.05$

morpho-physiological traits may be significant *in toto*. From Table 1, it is evident that significant variability is available in leaf length which ranged from 12.83 cm in Monty to 16.43 cm in Tomuri. Similarly, leaf width varied from 12.33 cm in Monty to 16.47 cm in Tomuri. Minimum petiole length i.e., 7.23 cm was found in Hayward and maximum i.e., 13.43 cm in Monty. Petal length was found to range from 2.13 cm in Tomuri to 3.03 cm in Monty. Minimum petal width was found in Allison i.e., 1.63 cm and maximum in Monty i.e., 2.24 cm. Flower diameter which is a measure of flower size, varied from 4.77 cm in Tomuri to 6.51 cm in Monty. It is important to study variability of floral parts such as petiole length, petal length, petal width, flower diameter etc. because flower organs contribute in the development of fleshy fruits. These desirable characteristics have led to a long history of selection, commercial development and understanding of fruit crops. Many crops bear fruit with little resemblance to their wild relatives because of long period of domestication. In contrast, all the cultivated kiwifruit species are only one or two generations removed from their wild relatives (Ferguson, 2007) which provides ample opportunity for further improvement through breeding to develop new varieties having desired morphological traits and high yield.

Table 2 shows that days to bud burst ranged from 115 in Monty to 127 in Hayward. Similarly, days to end bud burst ranged 126 in Monty to 131 in Hayward. Fruit length was found to vary from 5.70 cm in Monty to 6.17 cm in Hayward. As well, fruit width was found to vary from 3.50 cm in Monty to 4.73 cm in Hayward. Fruit weight varied from 53.10g in Bruno to 69.17g in Hayward. Fruit volume ranged from 66.50 cc in Abbott to 88.30 cc in Hayward. Days to harvesting ranged from 285 in Allison to 322 in Hayward. Farmers, horticulturalists and researchers are aware of weather differences causing large variations in dates of plant events for example the date of bud burst, date of end bud burst and flowering. Therefore, available variation in these traits may be utilized for cultivation in different areas as per weather condition. Kiwifruit typically requires 25 weeks from anthesis to reach physiological maturity, the earliest time at which fruit can be picked and continue to ripen satisfactorily (Beever and Hopkuk, 1990) but it may vary in different varieties and in different climatic conditions. Variability in fruiting traits is desirable for fetching higher returns.

Flowers, leaves, fruits and roots of plants contain numerous antioxidants such as vitamins, flavonoids and phenolic compounds. Antioxidants are considered important nutraceuticals on account of many health benefits (Valko *et al.*, 2007). Fig. 1 depicts that Monty variety had minimum phenol content *i.e.*, 106.18 mg $100g^{-1}$ f.wt. and Allison had maximum value of phenols i.e., 156.18 mg $100g^{-1}$ f.wt. These phenolic compounds have beneficial effect on health including anti-inflammatory, antiviral, antimicrobial, and antioxidant activity (Sree *et al.*, 2014). Fig. 2 gives a picture of variability in total flavonoids content of different varieties. Hayward variety had minimum flavonoids i.e., 120.08 mg $100g^{-1}$ f.wt. while Allison had maximum i.e., 31.47 mg $100g^{-1}$ f.wt. Flavonoids have the power to neutralise unstable molecules called 'free radicals' and these are valuable markers in chemotaxonomy.

Fig. 3 illustrates ferric reducing antioxidant power of different varieties which ranged from 82.41 mg of ascorbic acid

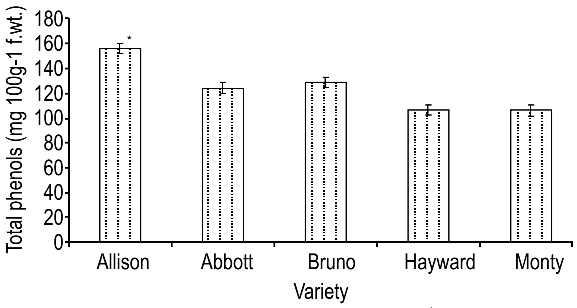

Fig. 1 : Variability in total phenol content (mg $100g^{-1}$ f.wt.) of different kiwifruit (*Actinidia chinensis* Planch) varieties. * Significant at $p \leq 0.05$

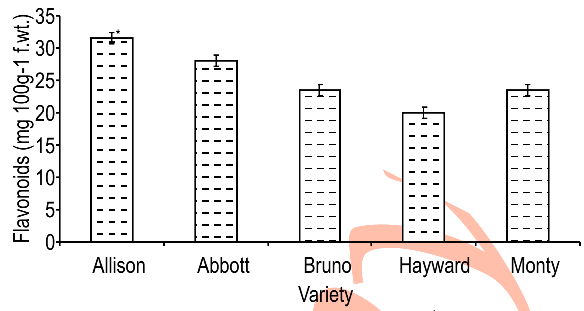

Fig. 2 : Variability in flavonoids content (mg $100g^{-1}$ f.wt.) of different kiwifruit (*Actinidia chinensis* Planch) varieties. * Significant at $p \leq 0.05$

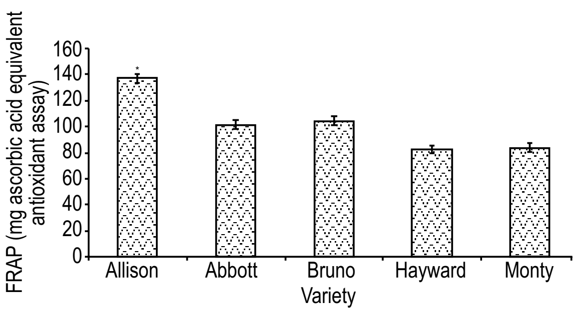

Fig. 3 : Variability in ferric reducing antioxidant power (FRAP) (mg of ascorbic acid equivalent antioxidant assay) of different kiwifruit (*Actinidia chinensis* Planch) varieties. * Significant at $p \leq 0.05$

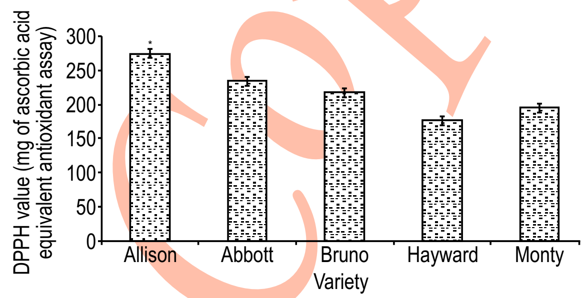

Fig. 4 : Variability in DPPH value (mg of ascorbic acid equivalent antioxidant assay) of different kiwifruit (*Actinidia chinensis* Planch) varieties. * Significant at $p \leq 0.05$

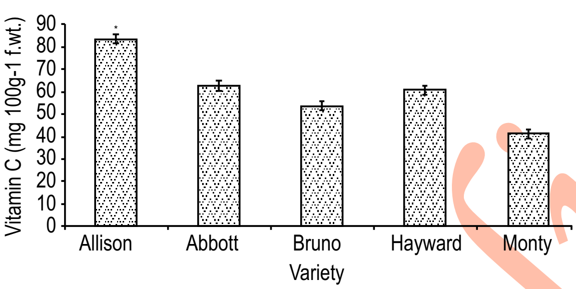

Fig. 5 : Variability in Vit. C (mg $100g^{-1}$ f.wt.) of different kiwifruit (*Actinidia chinensis* Planch) varieties. * Significant at $p \leq 0.05$

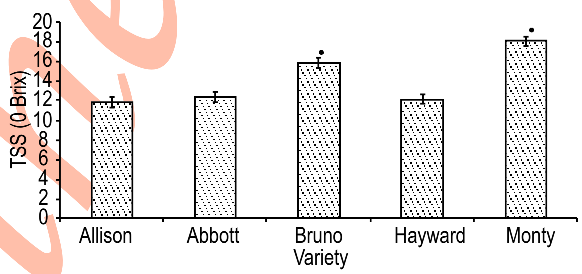

Fig. 6 : Variability in TSS (^{0}Brix) content of different kiwifruit (*Actinidia chinensis* Planch) varieties. * Significant at $p \leq 0.05$

equivalent antioxidant assay in Hayward to 137.19 mg of ascorbic acid equivalent antioxidant assay in Allison. Furthermore, it is clear from Fig. 4 that as per 2,2-diphenylpicrylhydrazyl (DPPH) assay minimum antioxidants were available in Hayward variety which was 176.32 mg of ascorbic acid equivalent antioxidant assay and maximum in Allison i.e., 274.97 mg of ascorbic acid equivalent antioxidant assay. Antioxidants in kiwifruit are more easily absorbed by the body than those in red grapes and strawberries (Prior *et al*., 2007), hence kiwifruit is more beneficial to health.

Fig. 5 shows variability in ascorbic acid (Vitamin C) contents of different varieties of kiwifruit. Minimum Vitamin C content was found in Monty variety i.e., 41.30 mg $100g^{-1}$ f.wt. and maximum in Allison i.e., 83.60 mg $100g^{-1}$ f.wt. Antioxidant-dense foods, like kiwifruit, are increasingly gaining popularity with consumers who look for health via natural and functional foods in addition to medicine. In recent years, more attention has been paid to antioxidants contained in fruits because epidemiological studies have revealed high fruit intake to be associated with reduced mortality and morbidity of cardiovascular disease and

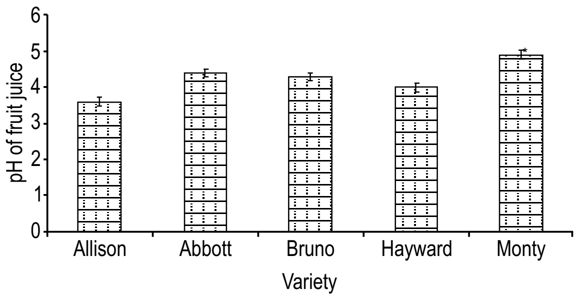

Fig. 7 : Variability in pH of fruit juice of different kiwifruit (*Actinidia chinensis* Planch) varieties. * Significant at $p \leq 0.05$

some types of cancer, possible mechanisms involves antioxidant activity present in fruits (Guo and Yang, 2001).

Total soluble solids in ready to eat fruits ranged from 11.85 °Brix in Allison to 18.05 °Brix in Monty (Fig. 6). Kiwifruit soluble solids continue to increase for several weeks after commercial harvest with simultaneous increase in the concentrations of fructose, glucose, and sucrose (Reid *et al.,* 1982). Analysis of fruit juice indicates that pH of fruit juice of Allison variety was highly acidic, pH value being 3.6 and that of Monty was least acidic in which pH value was 4.9 (Fig. 7). Juicy flesh of kiwifruit is slightly acidic and vary cultivar to cultivar (Ferguson, 1984). Total solids content of kiwifruit could be used to predict the ripe fruits and consumer acceptance (Mitchell, 1990). Comparison of different varieties showed considerable variability in form, fruiting characters and antioxidant activity. Kiwifruit contains many antioxidants and that ingestion of antioxidants decreases the death rate from cancer and cardiac disorder by reducing oxidative stress (Heber 2004). Previously, health benefits of kiwifruit established during *in vitro* research (Jung *et al*., 2005) were also confirmed during *in vivo* (Duttaroy and Jorgensen, 2004). Out of the 27 most commonly eaten fruits, kiwifruit has been recognised as the most nutritious (California Kiwifruit Commission, 2000). As a result, this fruit came to be known as the 'healthy fruit'. Among all the five kiwifruit varieties, Allison variety has been identified as the richest source of antioxidants. Therefore, the available variability in vegetative characters, flowering behaviour, fruiting attributes as well as antioxidant potential of different varieties might be harnessed and utilized for research and commercial applications. In view of the gradually decreasing trend of chilling hours in different areas of the Central Himalayan Region there is a need to find out low chilling fruit crops like kiwifruit. Moreover, morpho-physiological evaluation will be a crucial prerequisite to tailor varieties suitable for different regions. Now a comprehensive genetic map of 29 chromosomes of *A. chinensis* (Fraser *et al*., 2009), a considerable number of ESTs (Crowhurst *et al*., 2008) and draft genome (Huang *et al*., 2013) is available which provides ample opportunity for its improvement.

Among all the five kiwifruit varieties, Allison variety has been identified as richest source of antioxidants. Therefore, available variability in vegetative characters, flowering behaviour, fruiting attributes as well as antioxidant potential of different varieties might be harnessed and utilized for research and commercial applications. Besides having immense potential for cultivation, kiwifruit is not popular among fruit growers and comparatively less researched in the country. Hence, this might be used in breeding programmes to tailor genotypes having desirable vegetative and reproductive traits to meet out increasing demand of natural source of antioxidants and to cater the need of suitable genotypes for climate resilient horticulture.

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