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BIOCHEMICAL CHARACTERIZATION OF ONION (*Allium cepa* L.) GENOTYPES: IMPLICATIONS FOR BREEDING HIGH-NUTRIENT VARIETIES

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ABSTRACT

Numerous onion varieties are cultivated in India, exhibiting variations in antioxidant levels due to genetic and environmental factors. The health benefits associated with these antioxidant compounds motivate breeders to identify genotypes with superior functional properties. In the present study, we have attempted to characterize variations in biochemical compounds in different onion genotypes. Significant variations (p≤0.05) were observed in the phenolic content, anthocyanins levels, and activities of biosynthetic enzymes [(phenylalanine ammonia lyase (PAL), tyrosine ammonia lyase (TAL)] among thirty onion genotypes grown under sub-tropical Indian conditions. Among the red genotypes, namely D-305-B, Rec-1404, and PRO-6, high phenolic content, with a mean exceeding 0.7 g/100g DW, was observed. Genotypes Rec-1404 and PRO-6 exhibited the highest mean flavonol and ODH levels. Yellow genotypes POH-4 and POH-5 displayed high quercetin content. Anthocyanins levels were notably high in genotypes PRO-6 and Pb. Naroya. The highest PAL and TAL activities were recorded in the red genotypes D-888-B and Rec-1404, with mean activities exceeding 1.25 units. Total phenols exhibited positive correlations with ODH (r=0.902), flavonols (r=0.862), and anthocyanins (r=0.571). Additionally, TAL enzyme activity showed positive correlations with total flavonols (r=0.493) and anthocyanins (r=0.446). Overall, the red genotype PRO-6 emerges as a promising candidate for breeding programs aimed at developing onion varieties with high phenolics content. By prioritizing antioxidant-rich red genotypes and emphasizing phenolics content as a key parameter, breeders can contribute to the development of onions with enhanced health-promoting properties.

Keywords: Biosynthetic compounds, Onion, Phenolics, Phytochemicals

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Onion (*Allium cepa* L.) is a widely cultivated vegetable belonging to the Alliaceae family and is grown across temperate, tropical, and sub-tropical regions worldwide. In India, this biennial crop is cultivated twice a year during the *rabi* and *kharif* seasons. Onions are consumed, both raw and processed, as a component in salads and spices, at various developmental stages, from immature to mature bulbs (Chakraborty *et al.*, 2022).

Known for their distinctive taste and aroma, onions also boast diverse medicinal and nutritional properties. They are rich sources of biologically active compounds, including flavonoids, phenols, flavonols, anthocyanins, and phenolic acids (Zhang *et al.*, 2016). Phenolic compounds, a class of secondary metabolites abundant in many fruits and vegetables, include flavonoids, among other constituents. Flavonoids, further categorized into flavonols, flavones, isoflavonoids, and anthocyanidins, are synthesized within plant cells through metabolic pathways like the pentose phosphate, shikimate, and flavonoid pathways. These pathways are regulated by enzymes such as phenylalanine ammonia lyase (PAL), tyrosine ammonia lyase (TAL), chalcone synthase (CHS), flavonol synthase, and anthocyanidin synthase (ANS) (Khalid *et al.*, 2019).

Onions have been recognized for their numerous health benefits, including the reduction of cholesterol and blood sugar levels, as well as exhibiting antibacterial, antifungal, and antiparasitic properties (Teshika *et al.*, 2019). They also show promise in protecting the liver and containing anti-tumor components, attributed to the presence of over 200 chemical compounds (Asemani *et al.*, 2019). Moreover, fresh onions are noted to offer superior protection against infections and free radicals compared to processed onions. Consequently, onions hold significant potential for promoting health and preventing various illnesses.

Different onion varieties are cultivated across various regions of India, each exhibiting genetic differences among genotypes. Thus, the study aimed to assess the phenolic compounds and anthocyanins in thirty onion genotypes, alongside identifying the enzyme activities of phenol biosynthetic enzymes. These findings are crucial for identifying the most promising genotypes with significant health promoting properties.

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MATERIALS AND METHODS

Raising of the crop and procurement of samples

Thirty onion genotypes were cultivated at the Vegetable Research Farm, Department of Vegetable Science, Punjab Agricultural University, Ludhiana, India. The onion seeds were sown in November and subsequently transplanted into open fields in January. Upon reaching maturity, bulb samples were collected and analyzed for various biochemical parameters.

Chemicals

All chemicals and standards utilized were of analytical grade and procured from Sigma and Sisco research laboratories, Mumbai, India.

Estimation of enzyme activity

Estimation *of phenylalanine ammonia lyase activity (PAL)*

Phenylalanine ammonia lyase (PAL) was extracted and its activity was assessed following the method outlined by Hadwiger and Schwochau (1971). Fresh bulbs (100 mg) were extracted using 0.5M potassium phosphate buffer (pH 8.0) containing polyvinylpyrrolidone (PVP) at 4°C. Enzyme activity was measured using a spectrophotometer at 290 nm in the presence of reaction buffer (0.05M boric acid borax buffer pH 9.0) and substrate phenylalanine after one hour of incubation at 37°C. PAL activity is expressed as µmole of t-cinnamic acid produced per minute per 100g of fresh weight (FW).

Estimation *of tyrosine ammonia lyase activity (TAL)*

Tyrosine ammonia lyase (TAL) was extracted and quantified following the method outlined by Burell and Rees (1974). The extracted enzyme was incubated with substrate tyrosine in 0.05 M boric acid borax reaction buffer (pH 9.0). The absorbance of the reaction mixture was measured at 310 nm after one hour of incubation at 37°C. TAL activity is expressed as µmole of p-coumaric acid produced per minute per 100g of fresh weight (FW).

Extraction and estimation of Anthocyanins

Anthocyanins in bulb scales were extracted and quantified following the method outlined by Rabino *et al.* (1977) with slight modifications. The absorbance of the extracted pigment was measured at wavelengths of 530 nm and 657 nm against a blank. The final concentration was determined using the formula $A_{530} - 0.33\ A_{657}$ and expressed as milligrams per 100 grams of fresh weight (FW).

Extraction and estimation of phenolic compounds

For the extraction of phenolic compounds, freshly harvested mature onion samples were first cut into pieces and dried at 60°C in an oven. Once dried, the samples were ground into a powder to increase the surface area for extraction. Subsequently, 100 mg of the powdered samples were refluxed with 80% methanol. The filtered extract obtained after refluxing was utilized for the estimation of total phenols, flavonols, ortho-dihydroxy phenols, and quercetin.

Estimation of total phenols

The estimation of total phenols was conducted by the Folin-Ciocalteu reagent method, as proposed by Swain and Hillis (1959). In this method, the methanolic extract was reacted with the Folin-Ciocalteu reagent and sodium carbonate. The absorbance of the developed color was then measured using a spectrophotometer at 760 nm against a reagent blank. The concentration of total phenols was determined using a standard curve prepared with catechol (ranging from 10 to 100 µg ml^{-1}) and expressed as grams per 100 grams of dry weight (DW).

Estimation of flavonols

The estimation of flavonols from the methanolic extract was carried out according to the method proposed by Balbaa *et al.* (1974). The developed color was measured spectrophotometrically at 420 nm against a blank. The concentration of flavonol was determined using rutin as a standard and expressed as grams per 100 grams of dry weight (DW).

Estimation of ortho-dihydroxy phenols (ODH)

The estimation of ortho-dihydroxy phenols was conducted following the method proposed by Nair and Vaidyanathan (1964). In this method, the absorbance of the reaction mixture was measured at 540 nm. The concentration of ortho-dihydroxy phenols was determined using catechol as a standard (ranging from 10 to 100 µg ml^{-1}) and expressed as grams per 100 grams of dry weight (DW).

Estimation of quercetin

The estimation of quercetin content was done using the method proposed by Kumar *et al.* (2015), with modifications involving preparative TLC. In this method, the extracted quercetin content was measured spectrophotometrically at 254 nm. The separated quercetin content was determined using quercetin as a standard (ranging from 10 to 100 ng ml^{-1}) and expressed as micrograms per gram of dry weight (µg/g DW).

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Statistical analysis

The data are presented as mean ± standard deviation for three replicates. Statistical analysis was performed using ANOVA (analysis of variance) with a completely randomized design in SAS version 9.3 (SAS Institute, Inc, 1992; Cary, NC, USA). Post-hoc comparisons were made using Tukey's test at a significance level of 5%. Furthermore, Pearson correlation coefficient analysis was done to assess the correlation between the studied parameters.

RESULTS AND DISCUSSION

This study revealed the occurrence of antioxidants in different onion genotypes. Study showed that onion is a rich source of phenols. Genotypic variation in biochemical parameters was observed in all studied genotypes. Along with this, the biosynthetic enzymes phenylalanine ammonia lyase and tyrosine ammonia lyase influenced the phenolic contents in studied genotypes.

Analysis of neutraceuticals

In the current study, the total phenols content exhibited significant differences among the studied genotypes, ranging from 0.37 to 0.97 grams per 100 grams of dry weight (g/100g DW). Variation in onion bulb color also influenced total phenols content, with red, yellow, and white onions showing ranges from 0.50 (POH-2) to 0.97 (PRO-6), 0.42 (POH-5) to 0.84 (D-97-B), and 0.37 (PW) to 0.62 (D-48-B, PW-731035) g/100g DW, respectively. The highest phenols content was recorded in the red-colored PRO-6 genotype (0.97 g/100g DW).

The mean total phenols content across all genotypes was 0.73 g/100g DW. One-way analysis revealed significant differences both among different onion colors and within genotypes (Table 1). Comparable results were found in a previous study by Cheng *et al.* (2013), where red and yellow genotypes exhibited total phenols contents ranging from 0.606 to 2.23 g/100g DW and 0.57 to 1.8 g/100g DW, respectively, which align with our findings. Additionally, studies by Lee *et al.* (2015) and Bystricka *et al.* (2015) reported similar levels of phenolic content in red and yellow onions, with red onions showing higher total phenols content compared to yellow varieties.

In the present study, lower phenols and flavonols contents were noted in white onion genotypes at maturity compared to red and yellow genotypes, which corroborates findings from Kaur *et al.* (2009). Their study similarly observed higher phenols and flavonoids content in ten Indian-grown white onion cultivars over two successive years. According to existing literature (Patil *et al.*, 1995; Marotti and Piccaglia, 2002), the polyphenolics content in onions is influenced by genetic factors, environmental conditions (such as temperature, light, availability of water and nutrients), agrotechnical practices, and post-harvest conditions.

The ortho-dihydroxy phenols (ODHs) content ranged from 0.11 to 0.33 grams per 100 grams of dry weight (g/100g DW). The highest ODH content was observed in the PRO-6 genotype (0.33 ± 0.01 g/100g DW), a red-colored genotype. White genotypes exhibited lower ODH values compared to red and yellow genotypes. The mean ODH value for all genotypes was calculated as 0.20 g/100g DW, with a critical difference of 0.059.

Flavonols content in the present study ranged from 0.14 to 1.03 g/100g DW, with the highest values observed in red onions (Rec-1404) and the lowest in white (Pb. White) colored onion genotypes (Table 1). Flavonols content variation with respect to color was evident among the genotypes, with a critical difference of 0.133. The average flavonols content for all genotypes was determined to be 0.62 g/100g DW. One-way analysis indicated a significant difference between the red and white genotypes. In a similar study, Pérez-Gregorio *et al.* (2010) reported that the studied white cultivars had significantly lower total flavonols content compared to the red cultivars. They observed approximately three times more flavonols content in red cultivars than in white ones.

In the present study, quercetin content exhibited significant differences within genotypes with respect to color. Yellow genotypes displayed the highest quercetin content, with levels 1.55-fold higher compared to white-colored genotypes and 1.34-fold higher compared to red-colored genotypes. Quercetin content ranged from 4.66 ± 0.49 to 15.91 ± 0.40 micrograms per gram of dry weight (µg/g DW), with the highest content observed in the POH-4 genotype (15.91 ± 0.40 µg/g DW), a yellow-colored genotype. The pattern of quercetin levels among onion genotypes corresponded to their color, with yellow onions exhibiting the highest levels, followed by red onions, and then white ones. Red and white colored genotypes displayed quercetin content ranging from 4.66 ± 0.49 to 10.55 ± 0.24 µg/g DW and 4.98 ± 0.35 to 6.39 ± 0.62 µg/g DW, respectively (Table 1). Contrary to our findings, Bystricka *et al.* (2015) reported that red onion varieties contain higher amounts of quercetin than yellow varieties.

Anthocyanins serve as the primary pigments responsible for color formation in onions, derived from leucoanthocyanins. In red-colored genotypes, anthocyanin content ranged from 50.66 ± 0.99 to 16.45 ± 2.71 milligrams per gram of dry weight (mg/g DW), while in yellow and white genotypes, it ranged from 16.52 ± 0.84 to 4.35 ± 1.77 mg/g DW and 6.43

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Table 1. Enzyme activities (PAL and TAL) and phytochemical content of various colored onion (*Allium cepa* L.) genotypes

Color	Genotype	PAL Activity (µmole of t-cinnamic acid/ min/ 100 g FW)	TAL Activity (µmole of p-coumaric acid/ min/ 100 g FW)	Total phenols (g/100 g DW)	Flavonols (g/100 g DW)	Ortho-dihydroxy phenols (g/100 g DW)	Quercetin (g/100 g DW)	Anthocyanins (mg/ 100 g FW)
Red	D-PS-121-B	0.83 ± 0.07 ^{lm}	1.14 ± 0.06^{defghi}	0.68 ± 0.02^{efghij}	0.52 ± 0.01 ^{lm}	0.20 ± 0.02^{efghij}	6.31 ± 0.18^{efg}	16.45 ± 2.71^{i}
	D-715-B	0.89 ± 0.05^{klm}	0.99 ± 0.05^{fghi}	0.70 ± 0.01^{defghij}	0.50 ± 0.01 ^{lm}	0.16 ± 0.00^{hijklm}	6.74 ± 0.10 ^{de}	27.54 ± 0.67^{defgh}
	D-305-B	1.14 ± 0.12^{fghijk}	0.80 ± 0.10^{ijkl}	0.85 ± 0.06^{abc}	0.70 ± 0.03^{defghi}	0.24 ± 0.04^{def}	5.07 ± 0.36^{hij}	23.84 ± 1.77^{fgh}
	D-266-B	1.45 ± 0.11^{abcde}	0.93 ± 0.07^{ghij}	0.83 ± 0.02^{abcd}	0.81 ± 0.03^{cde}	0.24 ± 0.01^{cde}	5.32 ± 0.31^{fghij}	24.64 ± 1.77^{efgh}
	65 B	1.26 ± 0.04^{defghi}	1.22 ± 0.12^{cdefgh}	0.73 ± 0.09^{cdefghi}	0.73 ± 0.05^{defgh}	0.18 ± 0.02^{fghijk}	8.53 ± 0.15 ^{c}	24.42 ± 1.86^{fgh}
	D-31-B	1.42 ± 0.11^{abcdef}	1.32 ± 0.11^{cdef}	0.74 ± 0.01^{cdefgh}	0.80 ± 0.07^{cdef}	0.21 ± 0.02^{defghi}	10.55 ± 0.24 ^{b}	27.47 ± 0.64^{defgh}
	D-4-10-B	1.36 ± 0.06^{bcdefg}	1.57 ± 0.13^{bc}	0.70 ± 0.09^{defghij}	0.78 ± 0.04^{cdef}	0.19 ± 0.04^{efghij}	4.66 ± 0.49^{ij}	29.93 ± 0.89 ^{cd}
	D-888-B	1.66 ± 0.09 ^{a}	1.28 ± 0.13^{cdefg}	0.59 ± 0.05^{ijk}	0.60 ± 0.02^{hijkl}	0.14 ± 0.01^{jklm}	6.20 ± 0.22^{efgh}	27.61± 0.57^{defg}
	Rec-1404	1.06 ± 0.11^{hijkl}	2.38 ± 0.21 ^{a}	0.89 ± 0.02^{ab}	1.03 ± 0.02 ^{a}	0.30 ± 0.01^{abc}	6.70 ± 0.39 ^{de}	29.64 ± 0.45^{cde}
	Rec-1410	1.04 ± 0.13^{hijklm}	1.70 ± 0.09 ^{b}	0.71 ± 0.01^{cdefghi}	0.83 ± 0.01^{bcd}	0.18 ± 0.01^{fghijk}	6.00 ± 0.52^{efghi}	37.11 ± 0.98 ^{b}
	PDR-1260	0.87 ± 0.09^{klm}	1.14 ± 0.11^{defghi}	0.88 ± 0.01^{ab}	0.75 ± 0.06^{defg}	0.32 ± 0.01^{ab}	10.14 ± 0.24 ^{b}	33.63 ± 0.54^{bc}
	PR-10-853	1.14 ± 0.08^{fghijk}	1.31 ± 0.10^{cdef}	0.62 ± 0.02^{ghijk}	0.50 ±0.05 ^{lm}	0.13 ± 0.01^{klm}	5.70 ± 0.28^{efghij}	28.84 ± 1.69^{cdef}
	PR-10-367	1.29 ± 0.10^{cdefghi}	0.90 ± 0.12^{hijk}	0.80 ± 0.08^{bcdef}	0.69 ± 0.09^{efghij}	0.23 ± 0.03^{defg}	6.17 ± 0.23^{efgh}	33.84 ± 2.32^{bc}
	PRO-6	1.39 ± 0.06^{abcdef}	1.48 ± 0.14^{bcd}	0.97 ± 0.05^{a}	0.96 ± 0.03^{ab}	0.33 ± 0.01 ^{a}	6.15 ± 0.18^{efgh}	50.66 ± 0.99 ^{a}
	Pb. Naroya	1.63 ± 0.11^{ab}	1.48 ± 0.13^{bcd}	0.71 ± 0.08^{cdefghi}	0.63 ± 0.05^{ghijkl}	0.18 ± 0.02^{fghijk}	6.72 ± 0.47 ^{de}	47.25 ± 0.37 ^{a}
	PDR-821	1.27 ± 0.08^{defghi}	1.09 ± 0.11^{efghi}	0.75 ± 0.02^{bcdefg}	0.67 ± 0.03^{fghijk}	0.23 ± 0.01^{defg}	6.07 ± 0.26^{efghi}	26.82 ± 1.77^{defgh}
	Rec-1417	0.97 ± 0.11^{jklm}	1.06 ± 0.17^{fghi}	0.73 ± 0.03^{cdefghi}	0.55 ± 0.02 ^{kl}	0.20 ± 0.01^{efghij}	5.79 ± 0.28^{efghij}	23.87 ± 0.93^{fgh}
	PR-305	1.52 ± 0.10^{abcd}	1.13 ± 0.08^{defghi}	0.78 ± 0.02^{bcdef}	0.73 ± 0.06^{defgh}	0.22 ± 0.01^{defgh}	6.18 ± 0.91^{efgh}	22.54 ± 1.08 ^{h}
	POH-1	1.57 ± 0.07^{abc}	1.45 ± 0.14^{bcde}	0.63 ± 0.07^{ghijk}	0.56 ± 0.07^{jkl}	0.15 ± 0.01^{ijklm}	6.45 ± 0.17^{def}	27.86 ± 1.18^{gh}
	POH-2	1.65 ± 0.07^{ab}	1.73 ± 0.11 ^{b}	0.50 ± 0.03 ^{kl}	0.39 ± 0.01^{mn}	0.15 ± 0.01^{jklm}	8.45 ± 0.28 ^{c}	23.12 ± 0.98^{gh}
	POH-3	1.51 ± 0.06^{abcd}	1.30 ± .11^{cde}	0.80 ± 0.04^{bcdef}	0.91 ± 0.03^{abc}	0.24 ± 0.01^{def}	7.56 ± 0.36 ^{cd}	22.90 ± 1.95^{gh}
Yellow	D-97-B	1.18 ± 0.07^{efghij}	2.33 ±0.19 ^{a}	0.84 ± 0.04^{abcd}	0.79 ± 0.03^{cdef}	0.27 ± 0.01^{bcd}	5.26 ± 0.36^{ghij}	16.52 ± 0.84^{i}
	D-30-B	1.09 ± 0.05^{ghijkl}	0.56 ± 0.04^{kl}	0.67 ± 0.06^{fghij}	0.52 ± 0.05 ^{lm}	0.14 ± 0.01^{jklm}	6.25 ± 0.23^{efg}	4.35 ± 1.77 ^{lm}
	PBY10-214	1.02 ± 0.07^{ijklm}	0.88 ± 0.08^{hijk}	0.81 ± 0.01^{bcde}	0.58 ± 0.03^{ijkl}	0.27 ± 0.01^{bcd}	6.75 ± 0.22^{de}	14.86 ± 0.54^{ij}
	POH-4	1.29 ± 0.11^{cdefghi}	1.23 ± 0.11^{cdefgh}	0.57 ± 0.02^{jk}	0.41 ± 0.02^{mn}	0.16 ± 0.01^{hijklm}	15.91 ± 0.40 ^{a}	6.81 ± 0.57^{kl}
	POH-5	1.32 ± 0.12^{cdefgh}	1.33 ± 0.11^{cdef}	0.42 ± 0.02^{l}	0.31 ± 0.08 ^{n}	0.12 ± 0.01^{lm}	11.05 ±0.10 ^{b}	10.28 ±1.00^{jk}
White	D-73-B	1.63 ± 0.08^{ab}	0.58 ± 0.04^{jkl}	0.60 ± 0.03^{hijk}	0.34 ± 0.04 ^{n}	0.18 ± 0.06^{ghijkl}	5.70 ± 0.16^{efghij}	1.10 ± 0.89 ^{m}
	D-48-B	1.02 ± 0.05^{ijklm}	0.45 ± 0.06^{l}	0.62 ± 0.03^{ghijk}	0.36 ± 0.04 ^{n}	0.19 ± 0.01^{efghij}	4.98 ± 0.35^{ij}	6.43 ± 0.27 ^{kl}
	PW-731035	0.77 ± 0.10 ^{m}	0.84 ± 0.07^{ijk}	0.62 ± 0.05^{ghijk}	0.36 ± 0.01 ^{n}	0.16 ± 0.01^{ijklm}	6.20 ± 0.47^{efgh}	2.83 ± 0.35 ^{lm}
	Pb White	1.14 ± 0.11^{fghijk}	1.02 ± 0.11^{fghi}	0.37 ± 0.03^{l}	0.14 ± 0.01 ^{o}	0.11 ± 0.01^{m}	6.39 ± 0.62^{efg}	4.78 ± 0.35 ^{lm}
Mean		1.25	1.23	0.703	0.62	0.20	7.00	22.43
CD (p≤0.05)		0.289	0.362	0.14	0.133	0.059	1.15	5.05

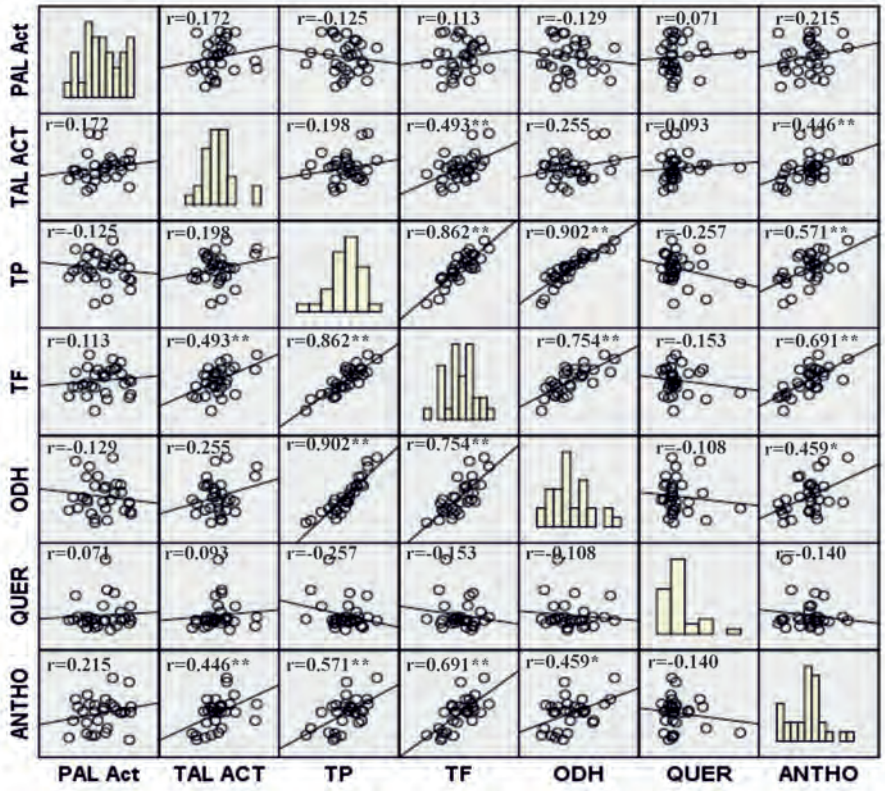


Fig. 1. Correlation between enzyme activities and different parameters studied in onion genotypes
***: Significant at p≤0.01, **: significant at p≤0.05**

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± 0.27 to 1.10 ± 0.89 mg/g DW, respectively. One-way analysis indicated significant differences among the studied genotypes. Interestingly, a significant range of anthocyanin content was observed within the same-colored onion group.

Numerous studies have reported that red onions contain higher anthocyanins than yellow and white varieties (Zhang *et al.*, 2016; Park *et al.*, 2018; Gonzalez-de-Peredo *et al.*, 2021). The variability in antioxidant contents such as anthocyanins and quercetin can be attributed to differences in cultivars and extraction methods (Prakash *et al.*, 2007).

Biosynthetic enzyme analysis

PAL enzyme activity plays a pivotal role in directing carbon flux from primary metabolism to the phenylpropanoid pathway (Ferrer *et al.*, 2008; Vogt, 2010). PAL is a crucial enzyme in the shikimate pathway, with its product serving as a substrate for subsequent enzymes, ultimately leading to the formation of specific flavonol glucosides such as quercetin or kaempferol-glucosides (Maeda and Dudareva, 2012).

In the present study, PAL activity was observed in the range of 0.77 to 1.66 units. The highest and lowest PAL activity levels were recorded in genotypes PW-731035 (red-colored) and D-888-B (white colored), respectively. Genotypes exhibiting higher PAL activity (Pb. Naroya, PR-305, D-266-B, POH-1, POH-2, and POH-3) were significantly different from one another and from the remaining genotypes. Statistical analysis indicated significant differences among all genotypes. The mean PAL activity value for all genotypes was determined to be 1.25 units, with a critical difference of 0.289. The data presented in the current study aligns with the findings of Mollavali *et al.* (2015) regarding mycorrhizal fungal infection in onions compared to control. They reported PAL activity in uninfected onions ranging from 0.15 to 3.5 units per milligram of protein.

TAL (Tyrosine ammonia lyase) is an alternative enzyme involved in synthesizing p-coumaric acid from tyrosine, regulating the phenylpropanoid pathway. To demonstrate the benefits of TAL activity in plants, the RsTAL gene was introduced into *Arabidopsis thaliana*. Over-expression of RsTAL led to the accumulation of flavonoid glycosides, confirming metabolic flux into the phenylpropanoid pathway (Nishiyama *et al.*, 2010).

In the present study, TAL activity ranged from 0.45 to 2.38 units, with the highest values observed in red and yellow genotypes (Rec-1404 and D-97-B) and the lowest in white (D-48-B) colored onion genotypes. Significant variations were observed with respect to both color and genotypes among the studied genotypes.

The average TAL activity among all genotypes was determined to be 1.23 units (Table 1).

Correlation coefficient of biochemical parameters

Figure 2 illustrates a significant maximum positive correlation (r=0.902) among all parameters recorded for total phenols and ODH, indicating a direct relationship between these parameters. Similarly, a strong positive correlation was observed between total phenols and total flavonols (r=0.862), as well as between total phenols and anthocyanins (r=0.571). Moreover, positive correlations were observed between total flavonols and ODH (r=0.754), total flavonols and anthocyanins (r=0.691), and between ODH and anthocyanins (r=0.459). Additionally, a lower positive correlation was observed between quercetin and all other parameters.

Remarkably, TAL enzyme activity exhibited a significant positive correlation with total flavonols (r=0.493) and anthocyanins (r=0.446). This finding suggests that enzyme activity is associated with the accumulation of flavonols and anthocyanins. Fahmideh *et al.* (2019) reported a strong positive correlation (r=0.759) among flavonoids and phenolic compounds. Furthermore, previous studies have supported these results, showing that PAL and TAL enzyme activities strongly correlate with anthocyanins and other flavonol synthesis (Sharma *et al.*, 2022).

The results indicate significant intergenotypic variation in antioxidant compounds, suggesting that many colored genotypes could serve as potential sources of antioxidant compounds with health-beneficial properties. Among these, PRO-6 (a red genotype) stands out as a promising candidate due to its high phenolics content. With its abundant yield of phytochemical components, PRO-6 could be considered a valuable genotype for breeding and pharmaceutical purposes.

Authors' contribution

Conceptualization of research work and design of experiments (NC, IK); Execution of field/lab experiments and data collection (IK, NC, ASD); Data analysis and interpretation (IK); preparation of manuscript (IK, NC).

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