

ASSESSMENT OF GROWTH AND BIOCHEMICAL INDICATORS FOR DROUGHT TOLERANCE IN MAIZE CULTIVARS

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Abstract

Ten maize cultivars were assessed to evaluate their response for drought tolerance under 100%, 60%, and 50% field capacity. Genetic variation is the unique mechanism of maize genotypes that are either tolerant or susceptible to varying degrees of drought. The drought stress significantly reduced the growth properties of all maize cultivars. A significant decrease in growth and the amount of chlorophyll content was observed in maize cultivars grown under 50% field capacity. Conversely, hydrogen peroxide (H₂O₂), malondialdehyde (MDA) contents, total soluble sugars (TSS), total flavonoids, anthocyanin, and total phenolic contents all showed a significant increase under drought stress. The results of this study demonstrated that the Gohar-19 cultivar of maize was tolerant to drought stress particularly at 60% field capacity with minimum reduction in growth, while the Pak Afgchoi cultivar of maize showed sensitivity to various levels of drought stress with a significant decrease in all growth attributes. Maize cultivars MMRI-Yellow, Malika, Pak Afgchoi, Agaiti-2002, and FH-1046 were found to be sensitive to varying degrees of drought stress, other genotypes of maize, including Neelam, Sahiwal Gold, YH-5427, and AF-5101, were found to be medium tolerant to drought stress. The Gohar-19 was the most tolerant cultivar and maintained its chlorophyll contents and development under drought stress.

Key words: *Zea mays*, Water stress, Plant growth indicators, Secondary metabolites.

Introduction

Maize is the principal cereal crop that is produced most worldwide. Maize production increases yearly but at a slow rate to meet the demands of food industry (Ashraf *et al.*, 2016). It is used as a feed and fodder crop both commercially and domestically due to its importance and main function in industrial crops. It also acts as a raw material to produce ethanol. The primary component of a plant body is water, which also serves as the primary cooling agent in plants and is essential to both plant evolution and expansion (Banziger *et al.*, 2000). The productivity and yield of maize is reduced by a variety of biotic and abiotic stress factors, including salt, drought, nutritional deficits, pest and insect problems, diseases, and extremes in temperature (Leach *et al.*, 2011). The most common of them is drought stress, which lowers the production of maize via a variety of mechanisms by influencing the plant's whole life cycle. Therefore, the productivity and production of maize is more severely affected by drought (Tahir *et al.*, 2022). One of the most obvious symptoms of drought stress is the decrease in the relative water contents. A fall in relative water contents causes a reduction in leaf water potential, which in turn causes stomata to become closer together (Farooq *et al.*, 2009). Elevated stomatal resistance leads to a decrease in transpiration rate, which in turn raises leaf temperature. Transpiration is the primary mechanism regulating leaf temperature; hence this is the primary cause of the increase in temperature (Arbona *et al.*, 2003). Plants have evolved a variety of water-retention methods to lessen the negative effects of drought stress such as reduction in transpiration rates and enhancing their capacity to extract water from the soil (Vegh, 2013). Additionally, plants use a variety of mechanisms or adaptation processes,

including chemical synthesis, accumulation, root system modification, stomatal modulation, and osmotic adjustment (Chaves *et al.*, 2003). Development of maize genotypes that are tolerant to drought stress is the greatest strategy to reduce the risk of harvest loss in maize crop under drought stress. A 15% increase in maize yield has been observed since drought-tolerant genotypes have been developed. Furthermore, compared to genotypes that are drought-sensitive, there is a 30% reduction in the risk of harvest failure (Simtowe, 2019). The development of drought-tolerant maize genotypes permits the development of kernels under drought stress by efficient use of water (Blum *et al.*, 2009). There are notable hinderances facing the genetics and breeding study, related to this subject matter (Araus *et al.*, 2012). In ecosystems facing drought stress, the inheritance of yield and other agronomic qualities is lowered; as a result, the features might not exhibit in subsequent generations (Lopes *et al.*, 2011). One recommended strategy is the ongoing selection of genotypes based on several traits associated with drought resistance (Banziger *et al.*, 2006). Maize crops need heat and water in the right amount and in a balanced manner to produce a higher yield (Chai *et al.*, 2022). Water shortage greatly affects maize yield, or rather, we may argue that water is the fundamental factor influencing maize production. Considering realities, the current study is designed to explore the drought tolerance potential of maize genotypes based on growth and biochemical indicators (Liu *et al.*, 2022). So, the main objective of the current study was to inspect key physio biochemical indicators for selecting potential commercial maize cultivars for drought tolerance, as cultivars tolerant to drought suggested for cultivation on drought-prone areas to produce more yield as compared to drought sensitive ones.

Material and Methods

The research work was carried out in the experimental area of Government College University Faisalabad, (GCUF). Seeds of the maize cultivars (Gohar-19, Sahiwal Gold, Malka-2016, Neelam, MMRI-Yellow, Pak Afghoi, YH-5427, Agaiti-2002, FH-1046, and AF-5101) were acquired from the Maize & Millet Research Institute (MMRI), Yusafwala, Sahiwal. The pots were filled with 8 kg soil and seeds of each variety were sown in it; the design used was completely randomized design (CRD) with three replications. Seven-day-old plants were then subjected to three different intensities of drought stress: 100% control, 60%, and 50% field capacity.

Growth attributes: After twenty days of germination, one plant was taken out of each pot to measure the growth parameters, including shoot and root length, fresh biomass of the shoot, and root. After allowing the plants to air dry, the dry weight of each shoot and root was measured in grams and plants were kept in an oven by adjusting at 65°C for 72 hours.

Biochemical attributes: Chlorophyll contents ("a", "b", and carotenoids) were measured using Arnon method (1949). Leaves were taken off to measure the amount of chlorophyll. About 0.1 g fresh leaves from each treatment were weighed and their chlorophyll content was measured by grinding the leaves in 80% acetone at 0.4°C. The solution was then left overnight and centrifuged at 10,000 rpm for five minutes to extract the supernatant. By a spectrophotometer (Hitachi-U2001, Tokyo, Japan), supernatant absorbance was measured at three distinct wavelengths: 645, 663, and 480 nm. The formulas were employed to analyze the chlorophyll contents as follows:

$$\text{Chlorophyll a} = [12.7 \times \text{OD } 663 - 2.69 \times \text{OD } 645] \times \text{Vol (ml)} / 1000 \times \text{wt. (g)}$$

$$\text{Chlorophyll b} = [22.9 \times \text{OD } 645 - 4.68 \times \text{OD } 663] \times \text{Vol (ml)} / 1000 \times \text{wt. (g)}$$

Kirk & Allen (1965) method was used for the determination of carotenoids.

$$\text{Carotenoids (mg ml}^{-1}\text{)} = \text{A.car/Em}100\% \text{ (Emission =2500)} * 100$$

Total phenolic contents: The total amount of phenolic contents in the leaf was calculated using the Julkenen-Titto (1985) method. About 0.5 g leaf material (fresh) grinded and homogenized in an 80% concentration of acetone solution for each replicate. After that, the material was centrifuged at about 10,000 x g for ten minutes to take the supernatant. About, 1 ml folin reagent and 2 ml of the plant extract was added to the mixture in the test tube. Added 20% of 5 ml sodium carbonate, to raise the total volume to roughly 10 ml. The solution was carefully homogenized. Finally, using a spectrophotometer, absorbance at 570 nm was measured.

Total soluble sugars: An 80% methanol solution was used for grinding a plant sample to estimate the total amount of soluble sugars. Added 3 ml of Anthrone reagent to 0.1 ml of plant extract material. Anthrone reagent was prepared by adding about 0.1 g of anthrone to

70% H₂SO₄. The solution in the test tube was placed in water bath for ten minutes. Mixture was left to stand for half an hour at room temperature. Readings were recorded at 625 nm (Yemm & Willis, 1954).

Flavonoid contents: Karadeniz *et al.*, (2005) devised the technique to determine flavonoid contents. About 1g plant leaves (fresh) weighed and grinded in 20 ml of 80% methanol. To obtain clear supernatant, the grounded material was then filtered. Added 0.3 ml (5%) of sodium nitrite, distilled water (3 ml) and 0.5 ml of filtered material. The mixture was placed at 25°C for about five minutes. About 0.6 ml of aluminium chloride and 2 ml (1M) sodium hydroxide was added in the mixture. The mixture was diluted by adding distilled water, with a final volume of 10 ml. Measurements were recorded at 510 nm. Using the standard calibration curve derived from rutin, the amount of flavonoids was calculated.

Malondialdehyde contents: Malondialdehyde contents were measured using the Cakmak & Horst (1991) method. Initially, 3ml of 0.1% (w/v) TCA solution was used to grind the one-gram leaf samples. Next, for 15 minutes at 20,000 rpm, the homogenized plant sample was centrifuged to extract the supernatant. A test tube was filled with approximately 0.5 ml of the top solution layer and 0.003 L of 5g/100 ml of TBA, yielding 20g/100 ml of trichloroacetic acid. The solution in the test tubes was allowed to boil in a water bath for about one hour. The water bath was kept at a temperature of 95°C. The samples were cooled and MDA concentration was measured at 532 and 600 nm wavelength.

H₂O₂ contents: The Velikova *et al.*, (2000) method was applied to determine H₂O₂ contents. About 6% TCA was used to crush the leaf material. Added 0.5 milliliters of the plant extract, mix it with 0.5 milliliters of K₃PO₄ buffer of 7 pH, and (1 mL, 1M) of potassium iodide. The measurements were recorded at 390 nm.

Total soluble protein: Bradford's (1976) technique was applied to calculate the total soluble protein. It was measured at 595 nm wavelength.

Anthocyanin contents: were measured by Mirecki & Teramura (1984). About 250 µl of acidic methanol was added, along with leaf sample. The plant sample was grinded in ice by using mortar and pestle and incubated at 4°C. The centrifugation of extract was carried out at 14,000 rpm for five minutes to get a clear supernatant. Finally, the measurement was recorded at 530 nm and 657 nm wavelength.

Total free amino acids: The amount was calculated by Hamilton & Van Slyke (1943) method. For this estimation, approximately 1 milliliter of plant extract was taken, and 1 milliliter of pyridine (1%) and 1 milliliter of nin-hydrin (2%) were added to the solution. The sample tubes were then submerged in a water bath and allowed to boil for 30 minutes at 95°C. Finally, using a spectrophotometer, measurements were recorded at 570 nm.

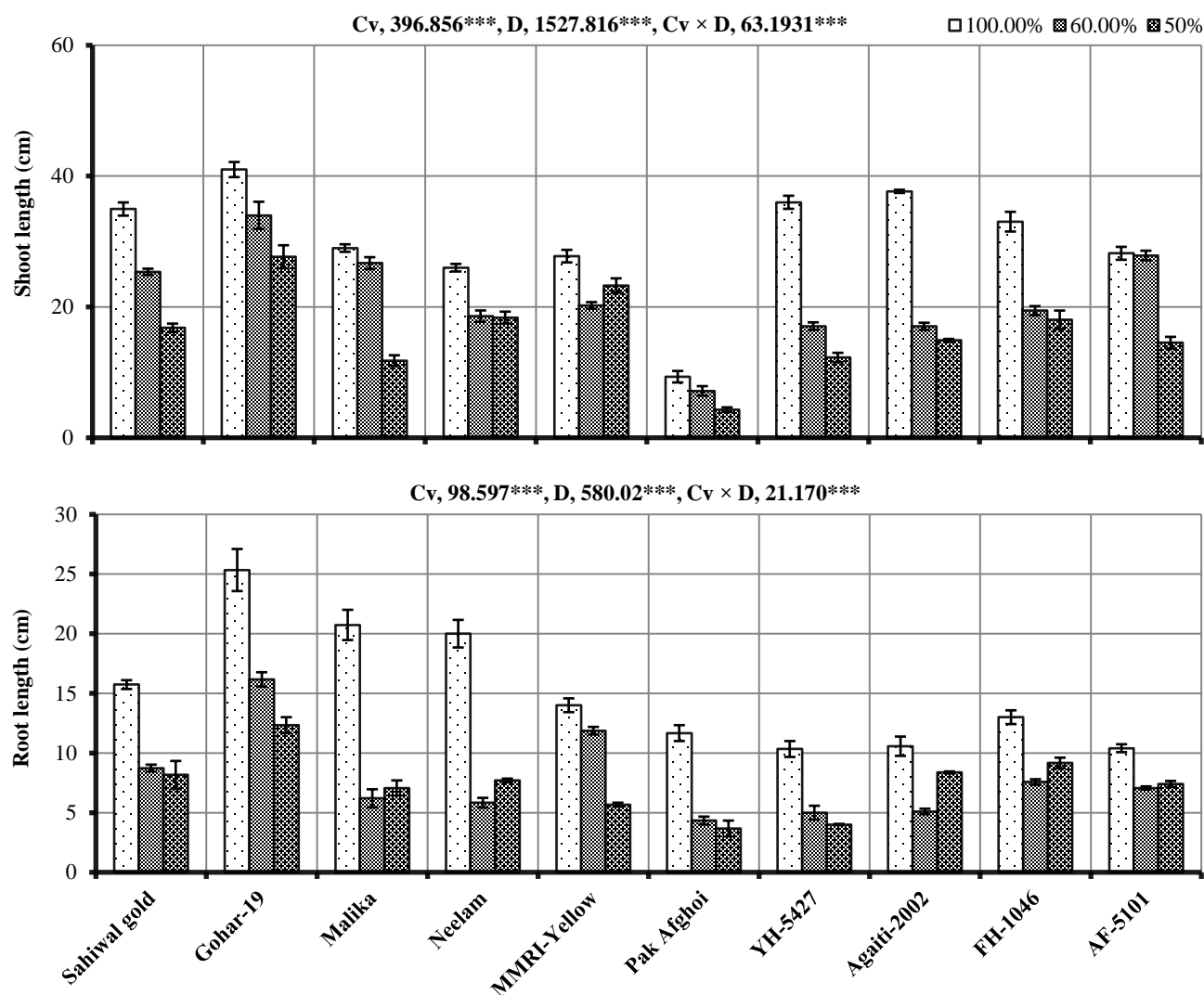


Fig. 1. The root length and shoot length of ten genotypes of maize (*Zea mays* L.) under drought stress.

Results

The root and shoot length was decreased with the increase in the degree of drought stress (Fig. 1). A discernible decline was noted in each cultivar of maize, with the greatest decline in Malika, MMRI-Yellow Pak Afghoi in all growth parameters. In contrast, cultivars YH-5427, Neelam, and AF-5101 exhibited resistance against drought stress, whereas MMRI-Yellow, Agaiti-2002, and FH-1046 showed a considerable decline in all growth parameters. Analysis revealed that under varying (100% control, 60% and 50% FC) drought stress conditions, all cultivars of maize (Gohar-19, Sahiwal Gold, Malika-2016, Neelam, MMRI-Yellow, Pak Afghoi, YH-5427, Agaiti-2002, FH-1046, and AF-5101) exhibited a decrease in both fresh and dry biomass of plant shoot as well as root (Fig. 2). In comparison to other maize cultivars, the Pak Afghoi cultivar had a notable decline in biomass (fresh & dry) of shoot, although the Gohar-19 cultivar exhibited the least amount of decline. The Sahiwal Gold, Neelam, YH-5427, and AF-5101 maize cultivars showed the least amount of decrease, whilst the Pak Afghoi cultivar showed the most drop. The data shown in (Fig. 3) indicated that, all maize cultivars cultivated under normal conditions showed a considerable rise in the concentration of chlorophyll

contents. Significant decrease was seen in chlorophyll a, b, and total contents with an increase in drought stress; however, the Pak Afghoi maize cultivar showed particularly, a considerable decrease. Under normal conditions, the concentration of leaf carotenoids was high in Gohar-19, which showed the largest rise in leaf carotenoids. All maize cultivars had a notable decline in leaf carotenoids with the increase in drought stress, however Pak Afghoi showed a particularly notable decline in leaf carotenoid levels when compared to other maize cultivars. All kinds of maize cultivars had low total anthocyanin contents when grown under controlled conditions, however all cultivars showed a notable rise in total anthocyanin contents with the increase in drought stress. Additionally, a notable rise was noted in the Sahiwal Gold cultivar of maize, whereas the FH-1046 cultivar showed the lowest level under 60% and 50% FC. Under drought stress, the concentration of total free amino acid increased noticeably. Total free amino acid content of all maize cultivars was increased; however, the Agaiti-2002 cultivar exhibited a greater increase in total free amino acid contents (Fig. 4). Under controlled conditions, quantity of total phenolic contents was remarkably high in all kinds of maize cultivars (Fig. 4). Conversely, as the level of drought stress was increased, a gradual rise in phenolic compounds was noted in

all kinds of maize cultivars. Comparing, the Gohar-19 cultivar exhibited a greater amount of total phenolic contents. Other cultivars that exhibited an increase in level of total phenolic contents are Neelum and FH-1046. However, with the increase in drought stress, all cultivars showed noticeable increase in total flavonoid contents, with the Malika showing higher levels than the others. All maize cultivars showed an increase in total soluble protein contents under controlled conditions, with the Gohar-19 cultivar exhibited the highest total soluble protein content. The concentration of total soluble protein was decreased with the increase in the intensity of drought stress. MMRI-Yellow exhibited the greatest decline in total soluble protein content, whereas Gohar-19 demonstrated the lowest reduction. Under normal circumstances, all maize cultivars had significant amount of total soluble sugar contents; however, as the degree of drought stress is elevated, the concentration of total soluble sugars in all maize cultivars increased gradually. Compared to other maize cultivars, Agaiti-2002 exhibited the highest increase in total soluble sugar contents, while MMRI-Yellow maize cultivar showed the lowest increase. Under controlled conditions, the concentration of H₂O₂ was variable in all cultivars of maize. However, when the degree of drought stress was increased, the Malika cultivar of maize exhibited the highest concentration of H₂O₂, whereas the AF-5101 showed the least value. Under controlled circumstances, the MDA content of the maize cultivars did not vary significantly. Nearly all maize cultivars had elevated MDA levels with the increase in drought stress level, YH-5427 and Pak Afghoi having particularly high MDA contents. However, a minimal increase was noted in the maize cultivar AF-5101 (Fig. 5).

Discussion

The findings of this study exhibited that water stress negatively impacted growth, productivity, physiological and biochemical attributes in all genotypes of maize. The growth characteristics were considerably reduced under conditions of water shortage. Restrictions in root structure, which limit the passage of water and nutrients for regular metabolic processes, may be the source of the decrease in maize growth brought on by water stress (Ali *et al.*, 2022). The three main processes that drive plant growth are differentiation, expansion, and cell division. Under water shortage, poor growth of plants was observed due to decrease in the process of mitosis and cell elongation (Hussain *et al.*, 2008). Lack of water restricts the growth of plant cell primarily owing to reduced turgidity (Taiz & Zeiger, 2006). Water restricting situations slow down cell growth mostly since there is not enough water flowing from the xylem to the adjacent cells. Shortage of water results in fewer and smaller leaves. Normally, the turgor pressure and assimilate supply determine how much a leaf expands. Abridged turgidity of cell and a slower photosynthetic rate under stress conditions are the main factors limiting leaf growth. Certain morphological traits, like plant length, biomass of both shoot and root are decreased under drought stress. Similar outcomes were reported by Zhao *et al.*, (2022). Under drought stress, all kinds of maize genotypes showed a considerable drop in their absorptions of pigment contents. Chlorophyll and particularly b were significantly impacted by drought stress. On the other hand, Sahiwal Gold, Gohar-

19, Malika, Neelum, and MMRI-Yellow maize genotypes showed a notable decrease in chlorophyll b. According to reports, plants under drought stress maintained larger concentrations of chlorophyll a than chlorophyll b (Jain *et al.*, 2010). Thylakoid membranes and photosynthetic pigments are damaged by drought (Anjum *et al.*, 2011). There have also been reports of decreased chlorophyll contents under drought stress (Din *et al.*, 2011). The most recent data showed a greater decline in chlorophyll-b, which suggests that some genotypes are more susceptible to drought stress. It has been reported that the photosynthetic pigments are reliable markers to determine the conflicting effects of drought stress. Similar to this, drought stress has a detrimental impact on plants' overall chlorophyll contents and gas exchange characteristics. In our research drought stress decreased the amount of total leaf carotenoids in all maize genotypes. Carotenoids play a variety of roles in drought tolerance, such as protection against oxidative damage is brought on by tolerance to drought stress and harvesting light. Consequently, the plant's metabolic system may suffer destruction if the carotenoid level is decreased. Our findings are consistent with those of Havaux (1998) and Kiani *et al.*, (2008), who found that drought stress decreased total leaf carotenoids and chlorophyll. Our findings correlate with that of Koutoua *et al.*, (2016), who found that drought reduced tomato plant height, carotenoids, and specific weight. Under limited water conditions, the anthocyanin contents of all genotypes of maize showed a considerable increase. In response to abiotic stresses such as drought, excessive salinity, light, and cold, plants produce anthocyanin, which are frequently associated with increased stress tolerance. Zhao *et al.*, (2022) and Cao *et al.*, (2022) established comparable results, indicating that anthocyanin concentration rises in response to drought stress. Under drought stress, maize genotypes exhibited an increase in total free amino acid content as reported by Ma *et al.*, (2016). Additionally, our results are comparable with Obata *et al.*, (2015) and Chmielewska *et al.*, (2016). Plants containing phenolic compounds have a number of secondary metabolites that are involved in preventing oxidative damages (Krol *et al.*, 2014) caused by stress and have antioxidant qualities. The total phenolic contents of plants can decrease or increase in response to stress conditions (Al Hassan *et al.*, 2015; Gharibi *et al.*, 2015). The total phenolic compound in all genotypes of maize decreased in our study, which was in accordance with findings from (Rivas-Ubach *et al.*, 2012 & Fraire-Velazquez & Balderas-Hernandez, 2013) and Weidner *et al.*, (2009), who reported total phenolic contents in grapevine roots under drought stress. According to our research, the total flavonoid content of all maize cultivars showed a considerable increase as the degree of drought stress increased. The outcomes of our research show similarity with Gao *et al.*, (2020), who found that drought prompted the accretion of secondary metabolites, for instance flavonoids, in two different Adonis species. Talbi *et al.*, (2020) indicated that antioxidant capacity and flavonoid accumulation in the Saharan plant *Oudeneya africana* increased under drought stress and also improving plant adaptation to abiotic stress. A change in total soluble protein is observed in all genotypes of maize under drought. Riccardi *et al.*, (1998) reported that total soluble proteins of two genotypes of *Zea mays* in the leaves and roots increased initially, then decreased under drought stress.

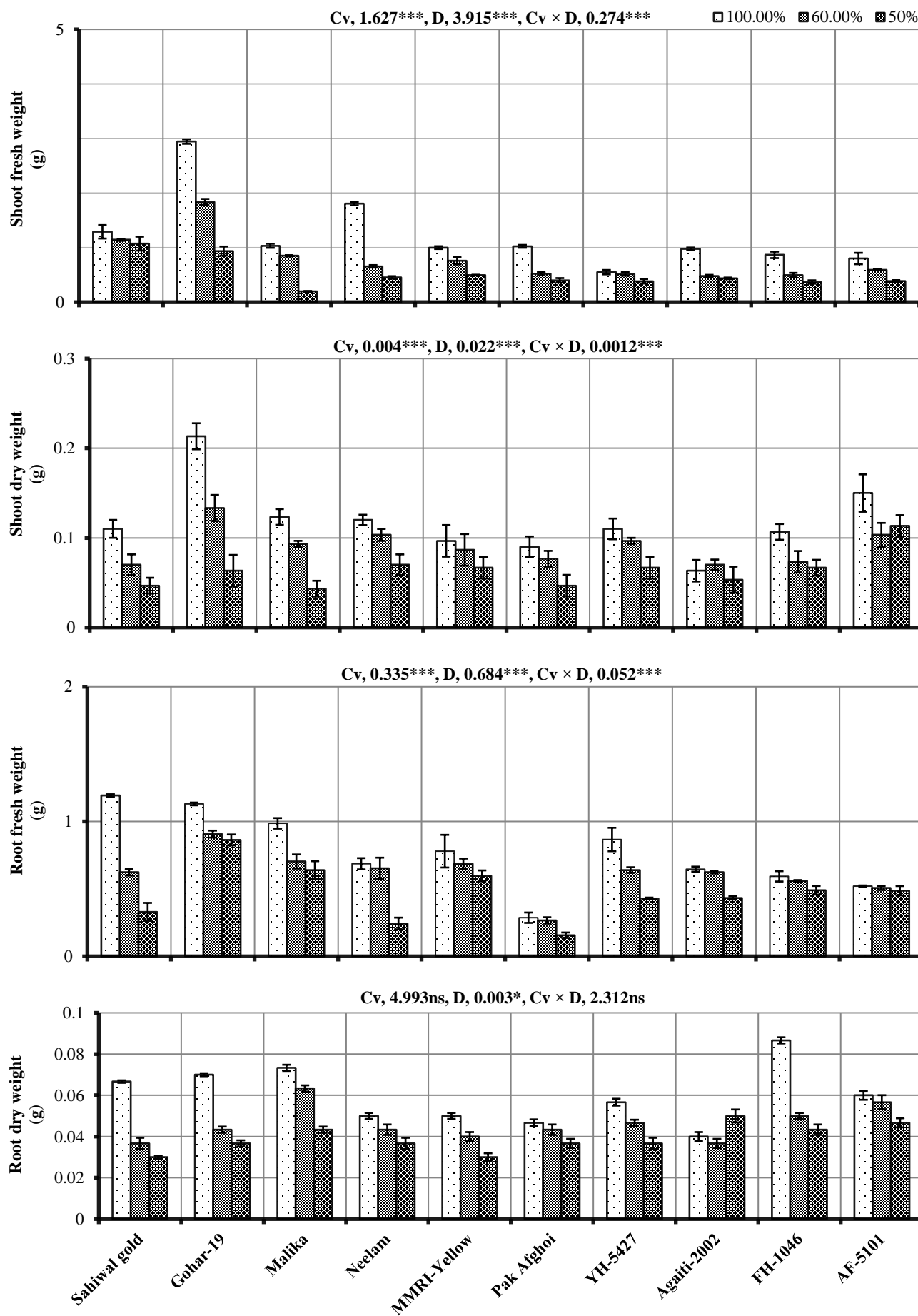


Fig. 2. The fresh and dry biomass of ten genotypes of maize (*Zea mays* L.) under drought stress.

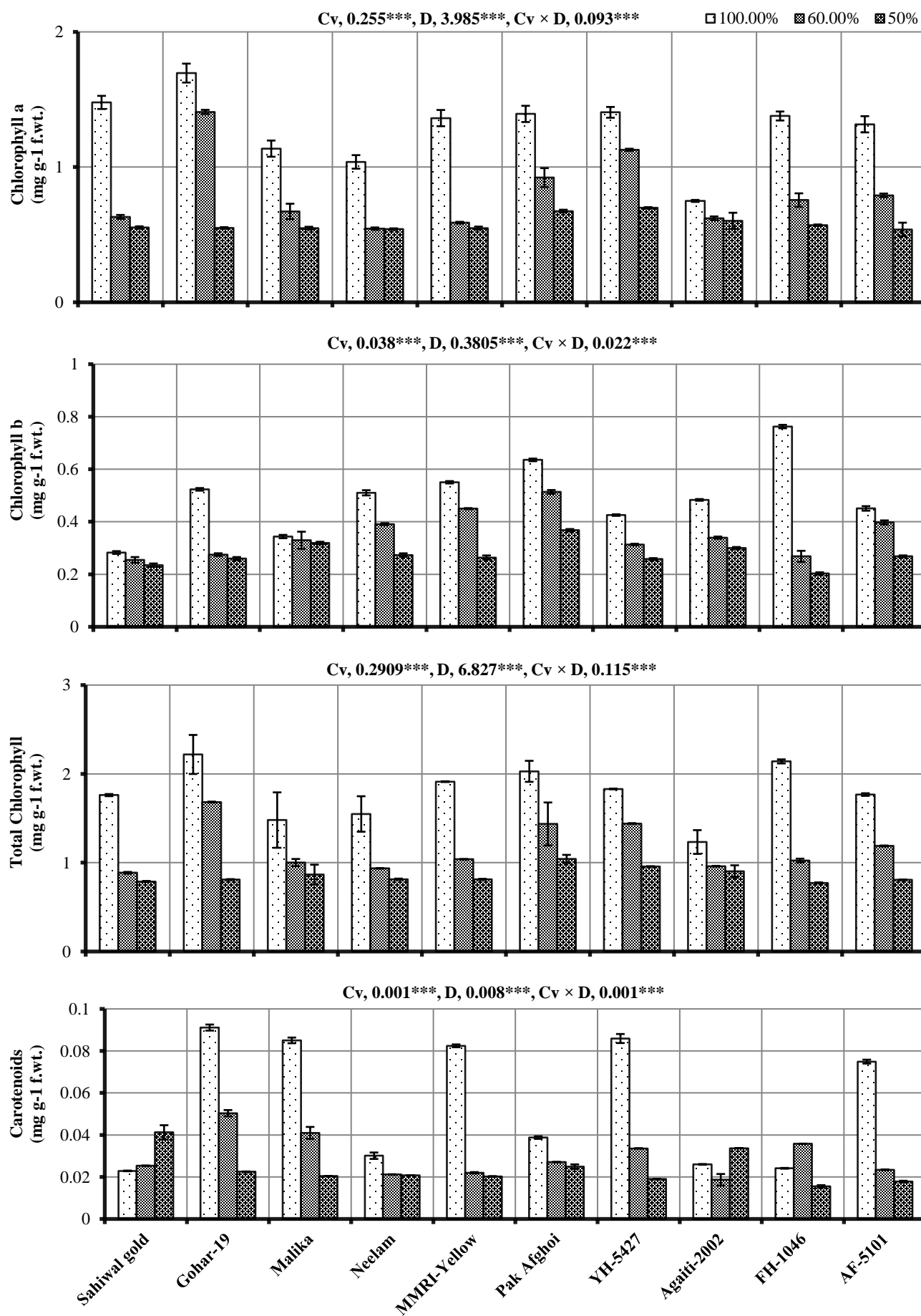


Fig. 3. The pigment contents of ten genotypes of maize (*Zea mays* L.) under drought stress.

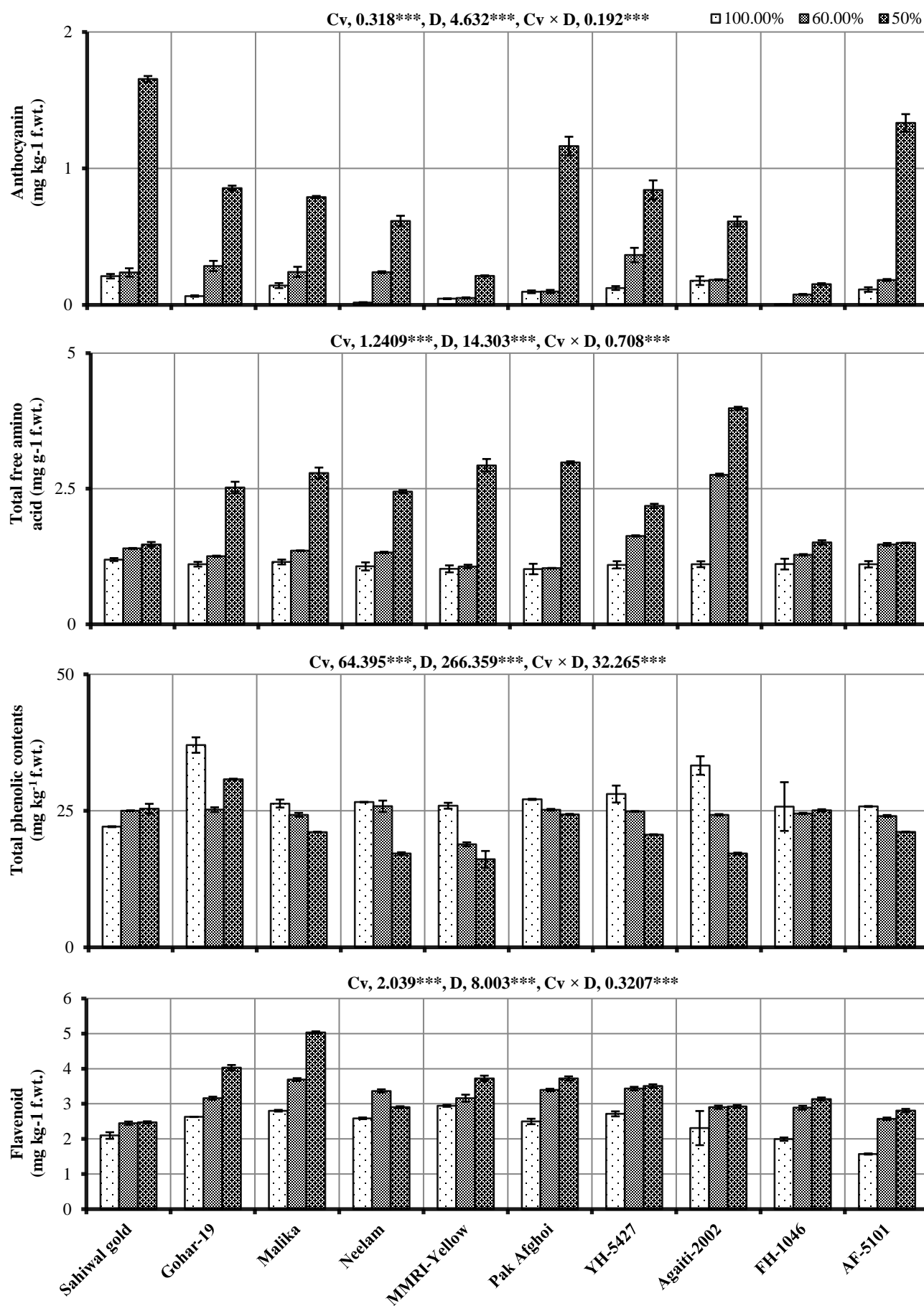


Fig. 4. The anthocyanin contents, total free amino acid, total phenolic contents and total flavonoids contents of ten genotypes of maize (*Zea mays* L.) under drought stress.

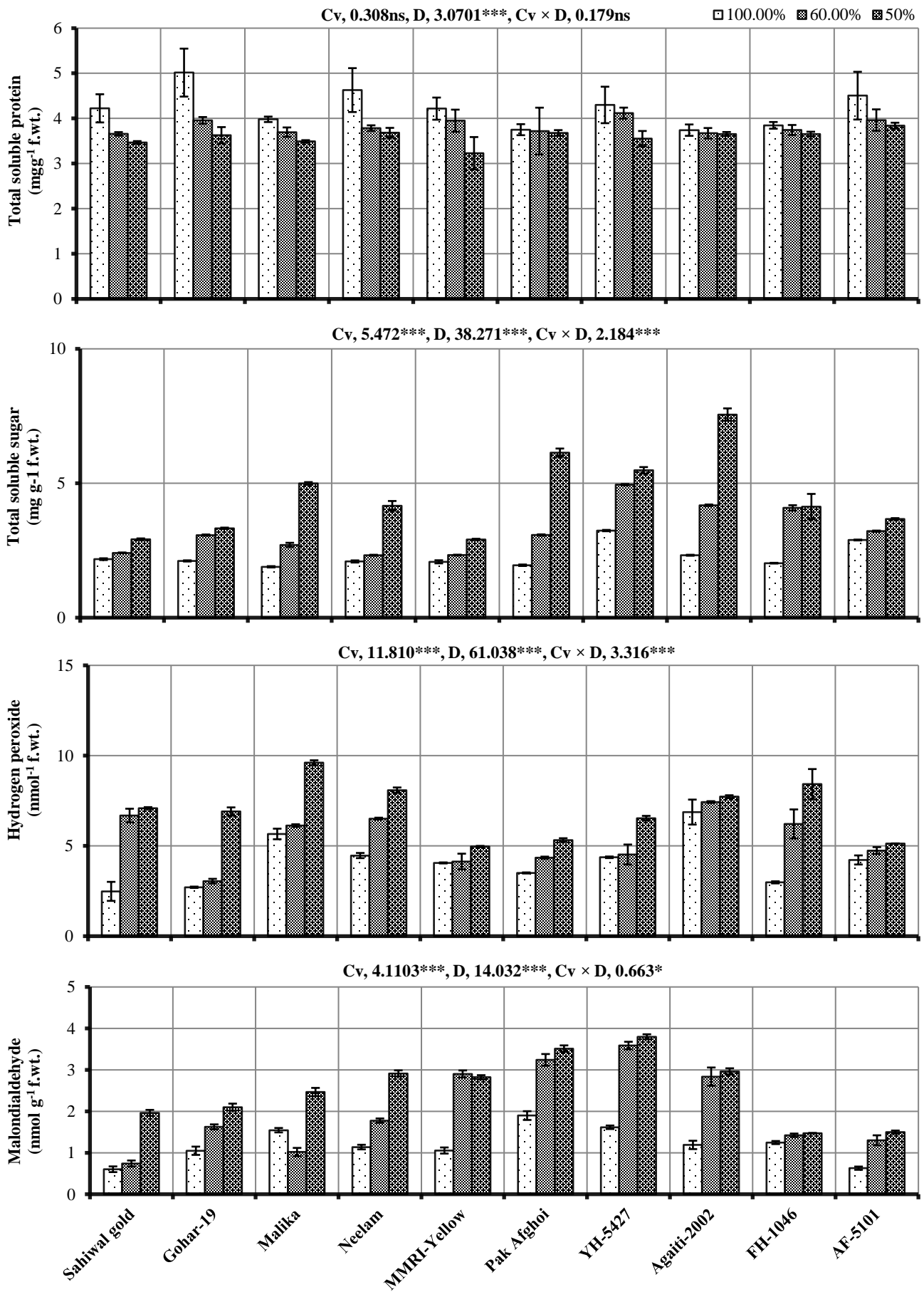


Fig. 5. The total soluble proteins, total soluble sugar, hydrogen peroxide and malondialdehyde contents of ten genotypes of maize (*Zea mays* L.) under drought stress.

All genotypes of maize showed an increase in total soluble sugar content when subjected to drought stress. These findings show resemblance to Sperdouli *et al.*, (2012). It is also well known that sugars accumulate in response to drought stress (Watanabe, 2000). It is commonly known that soluble sugars play a complicated and vital function in plant metabolism as byproducts of hydrolytic activities, substrates in biosynthetic processes, producers of energy, as well as in systems that sense and interact with sugar. According to recent claims (Kishor *et al.*, 2005), even sugar flow may serve as a signal for metabolic regulation when conditions are stressed due to drought. Additionally, soluble sugars can act as an ordinary osmo-protectant, keeping turgor pressure constant and stabilizing cellular membranes. As a byproduct of lipid peroxidation, MDA is frequently employed for assessing oxidative stress in situations of water stress (Farooq *et al.*, 2010). Drought is the major, of many abiotic stressors that causes creation of ROS, which damage the membrane in diverse ways. One such ROS is H₂O₂, it is reported that increased H₂O₂ content increase MDA concentration in plants under drought stress (Ashraf *et al.*, 2016). All kinds of maize genotypes showed a notable rise in MDA and H₂O₂ levels in response to drought stress

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