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Original Research Paper

Study of root anatomical modifications among susceptible and drought tolerant garlic (*Allium sativum* L.) genotypes by FESEM-EDS

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ABSTRACT

An experiment was conducted to demonstrate the root anatomy under control and drought stress in order to investigate the dynamics of drought among genotypes of garlic (susceptible and resistant genotypes). The resistant genotype of garlic had a more pronounced root epidermal layer arrangement without root collapse than the susceptible genotype. The distribution and content of elements were revealed by the Energy Dispersive X-ray Spectroscopy (EDS) spectra of the FESEM. The FESEM-EDS mapping spectra of the resistant genotype micrographs revealed that Cu, Mn, and Zn elements accumulated least during drought stress, while, Cl, Na, Si, Mo, and Ni elements accumulated most. Notably Zn and Co accumulation was observed in the tolerant line that was acquired under drought stress. In contrast, vulnerable genotypes tend to accumulate more Cl and Na than tolerant genotypes do. These results may contribute to a better understanding, characterization, and identification of genotypes resistant to drought stress through FESEM-EDS studies.

Keywords: Drought, EDS mapping, FESEM, garlic, morphology, root

INTRODUCTION

In the *Allium* genus, garlic (*Allium sativum* L.), is the second most popular farmed bulb crop after onion, belonging to the family *Alliaceae*. Despite being a native of central Asia, garlic was produced and consumed everywhere in the world, from temperate to subtropical climate (Fritsch & Friesen, 2002). Over time, both production and the area grown worldwide have been scaled up, the most garlic growing countries are China, India, the United States, South Korea, Egypt, Myanmar, Russia. Biotic and abiotic stress can cause a yield losses up to 70% (Kamenetsky, 2007; Kamenetsky et al., 2004; Nagakubo et al., 1993). Drought stress has been proven to be a crucial limiting factor throughout the plant growth and development stages. Under water-limited conditions, higher plant fresh and dry weights are desirable traits. A common negative impact of water stress on crop plants is a decrease in the production of fresh and dry biomass (Farooq et al., 2009; Jaleel et al., 2009). Programs for breeding plants mostly aimed to develop genotypes with high yields. However, the plant breeder's goal is oriented towards crop enhancement programs for reliable and sustainable productivity under variety of environmental conditions.

Plants absorb water and nutrients primarily through their roots, and they also have the extraordinary ability to sense and adapt to the majority of physicochemical conditions in the soil by changing their growth and water transport capabilities. As a result, it is essential to conserve the nutritional and developmental functions of the plant in the face of abiotic stresses. As a drought adaptation strategy, evaluating the ability of various root types to absorb water by comparing their differences root morphology is possible (Wasaya et al., 2018). Drought has evolved into a substantial adverse environmental impact as a result of climate change and global warming, posing a serious threat to agriculture. In the long run, the creation of crops that can withstand droughts is essential for global food security. Therefore, in order to develop drought-tolerant plants, especially crops, a thorough understanding of plant responses to drought stress is a requirement (Zhou et al., 2021). To contend from drought stress, plants exhibit a variety of physiological, biochemical, and molecular responses. Furthermore, it is well recognized that drought affects shoot growth far more than root growth, leading in a modified shoot: root ratio, the root system of a plant is more susceptible to abiotic stresses than its aerial parts (Franco et al., 2011). Thus, insights on root morphology and physiology aid to understand the drought resistance and susceptible genotypes.

The scanning electron microscopy (SEM) was utilized to study the structural variability of surface at a micro scale of plants in the family *Acantheacea* (Al-Hakimi et al., 2017). The relative composition and stoichiometry of minerals are revealed by the EDX spectra (Jadhav et al., 2016). To record the topography and distributions of the elements, FESEM image analysis and EDS Spectra was developed by mapping of elements. Analyses of pollen morphology by scanning electron microscopy techniques, and further revealed the elemental details of pollen in chives and Chinese chives by FESEM-EDS spectra upon mapping elements (Manjunathagowda et al., 2022a, 2022b). Garlic an economically significant monocotyledonous plant with shallow roots, and it is sensitive to water scarcity. In the most crucial time (seedling and bulbing stage), drought stress severely affect the crop growth and bulb yield. Therefore, investigation on drought stress in garlic genotypes which are resistant and susceptible to drought stress, was carried out.

MATERIALS AND METHODS

In the study, two garlic genotypes DOGR-28 and DOGR-24 were employed for FESEM-EDS assay at ICAR-Directorate of Onion and Garlic Research (DOGR), Pune, India during 2019 to 2021. The tolerant and susceptible germplasm lines were grown as per the package of practice recommended by ICAR-DOGR, Pune, India. Drought stress condition was imposed after 40 days of planting of cloves, where water was withhold for 25 days. The set of control plants was irrigated regularly as per the recommended package of practice of ICAR-DOGR, Pune. The plants were monitored frequently during this time. After completion of 25 days of drought stress, the root samples were collected from control as well as from drought stress induced treatments, root anatomy was analyzed by using Field Emission Scanning Electron Microscopy and Energy Dispersive X-ray Spectroscopy (FESEM-EDS).

The first step in preparing an object for SEM observation is to make it current conductive. This is accomplished by applying a gold layer that is between 1.5 and 3.0 nanometers thick. Additionally, objects must be able to withstand the high vacuum and shouldn't change it, like by losing gas or water molecules. Typically, crystals, metals, and polymers are a little troublesome and maintain their structure in the SEM.

FESEM: FEI Nova NanoSEM 450 Scanning Electron Microscope (Nova NanoSEMTM), with exceptional low vacuum capabilities and ultra-high resolution low voltage imaging. Images were examined by FESEM software xT microscope with a resolution of up to 1.0 nm at 15kV, 1.4 nm at 1kV, and 1.8 nm at 3kV and 30Pa by the In-lens TLD, SE, and BSE detection, which is equipped with Load lock (Quick Loader).

Additionally, the FEI Nova NanoSEM 450 Scanning Electron Microscope has a BrukerXFlash 6I30 EDS detector for elemental composition investigation. Control EDS Espirit 1.9 software was used to analyze images with excellent energy resolution of 123 eV at Mn K and 45 eV at C K and element detection range from 4 Be to 95Am.

RESULTS AND DISCUSSION

Root growth and development are essentially implicated in response to drought stress. In the study, the root anatomy and molecular parameters studied and discussed.

Root anatomy of transverse section under control and drought stress

The transverse sections of garlic roots revealed specific anatomical modifications such as formation of larger sized aerenchyma cells with cortex shrinkage were observed in tolerant (DOGR-24) and in susceptible (DOGR-28) garlic genotype under drought stress conditions (Fig. 1). Whereas, the root cortex collapsed and severely affected the internal root cells of susceptible cultivar under drought stress (Fig. 1d), which were revealed by field emission scanning electron microscope (FESEM) visualized the root sections at 200-300 µm (Fig. 1).

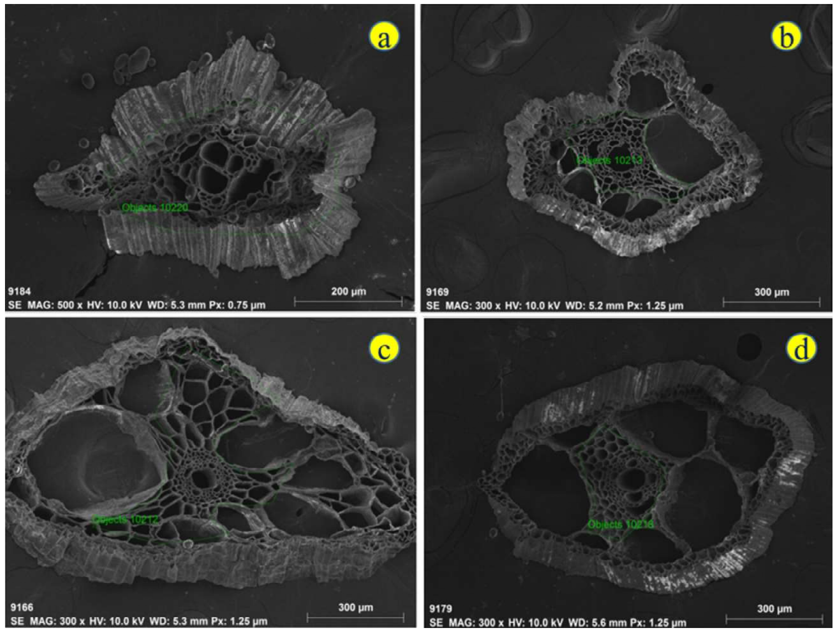

Fig. 1: Root morphology under control condition (a) and drought stress (b) of DOGR-24, and DOGR-28 roots revealed under control condition (c) and drought stress (d)

Differential expression of elements under control and drought stress

Root transverse section of genotypes DOGR-24 (resistant) and DOGR 28 (Susceptible) under control and drought stress induced samples were readily revealed the composition of atomic elements by FESEM-EDS spectra micrographs. The elements Ca, Mn, Zn, Si, Fen A, Co, Cu, Ni, Cl, Mo, and B were detected at a topography of 100-200 µm scale (Fig. 2).

The relative mineral compositions of genotypes DOGR-24 (resistant) and DOGR 28 (susceptible), under control and drought stress were varied for their relative percentage of elements Cl, Na, Mo, Si, Ni, Mg, Mn, Ca, Fe, Cu, S, B, Co, Zn, respectively found in the genotype DOGR-24, a drought resistant cultivar.

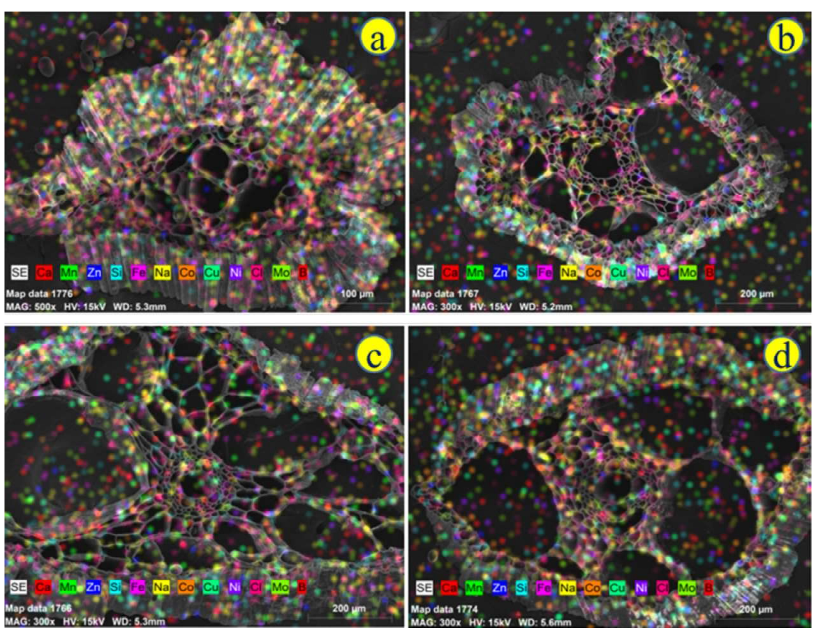

Fig. 2: Mapping of root transverse section for mineral composition under control condition (a) and drought stress (b) of DOGR-24, and DOGR-28 roots revealed under control condition (c) and drought stress (d)

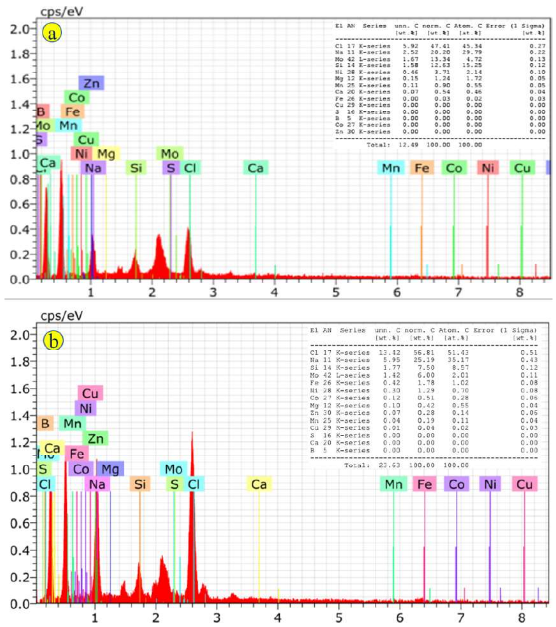

Fig. 3: Differential expression of elements upon mapping on root revealed by the genotype DOGR-24 under control condition *vs*. drought stress

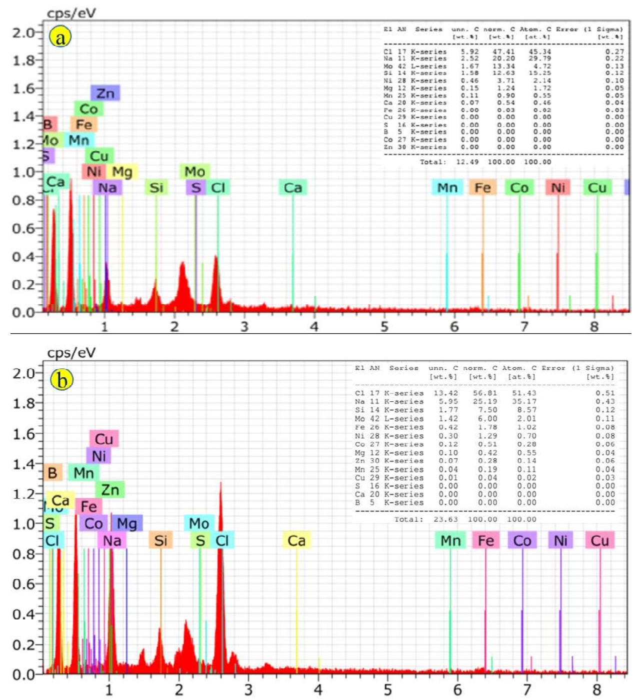

Fig. 4: Differential expression of elements upon mapping on root revealed by the genotype DOGR-28 under control condition *vs*. drought stress

The elemental relative percentage was noted lower in the controlled condition than in the drought stress condition (Fig. 3). However, Ca was observed under controlled conditions, but not in drought stress, whereas Cu, Mn, and Zn were recorded only under drought stress. Mn was present only in tolerant cultivars, and which is absent in susceptible cultivar under controlled and drought condition (Fig. 3, 4). In the genotype DOGR-28, the elements namely Cl, Na, Si, Co, S, Ca, and Mg witnessed lower percentage under controlled conditions than the drought stress condition expect Si, Sulfur (S) witnessed only under controlled conditions but not under drought stress (Fig. 4).

In response to drought stress, root development and their modifications is primarily involved. Under drought stress conditions, garlic plants adjust to number of factors mainly root cell organization and their modification. Tolerant and susceptible garlic genotypes (DOGR-24 and DOGR-28, respectively) under drought stress were compared with control (Fig. 1 & 2). The present experiment revealed that there are many root morphological parameters associated with the root modifications in garlic genotypes under drought stress. The transverse sections of garlic roots revealed specific anatomical modifications such as formation of least aerenchymatic cells with low cortex shrinkage in resistant cultivar and larger aerenchyma cells with larger cortex shrinkage were observed in susceptible garlic genotypes under drought stress, the FESEM image illustrations could aid for isolation of draught tolerant lines as a reference visual root transverse section data. However, Suralta & Yamauchi (2008) observed aerenchyma formation under drought conditions in both aerobic and lowland genotypes, indicated to be that plant trying to adopt to the drought by forming aerochyma cells, meanwhile, the collapse of cortex cells leads to susceptibility to drought stress. Sibounheuang et al. (2006) reported that xylem diameter is related to maintenance of xylem water conductivity, the collapse of xylem cells could lead to the susceptibility of crops to drought stress. Yambao et al. (1992)–put forward that modification in xylem vessels to be a useful trait for recuperating water absorption from soil depth. Further, there is need to correlate between architectural and anatomical traits, like number of cortex cells and metaxylem vessels in garlic roots under drought stress. However, Hazman & Brown (2018) were found positive correlation with lateral root length, but observed negative correlation with maximum root depth. Hazman & Brown (2018) reported that both the basal and apical segments of nodal roots altered their anatomical structure and composition in response to drought. The expansion in the root arrangements by plants to augment the water uptake to sustain growth under drought stress condition as observed in the present research study mainly in the tolerant cultivar (DOGR-24).

The FESEM-EDS offers topographical and elemental information with a practically infinite depth of field of magnifications at magnifications ranging from 10 to 300,000. FESEM creates images that are clearer, sharper, and less electrostatically deformed with spatial resolution down to the nano- or micro-scale (Fig. 2). Fine layer thicknesses, structural arrangements, structure homogeneity, minute contamination, feature geometry, and elemental composition information can all be displayed via FESEM analysis (FESEM, 2021; Jadhav et al., 2016). FESEM and FESEM-EDS have been used to reveal the morphological characteristics of the male gametophytes from the chive (*Allium schoenoprasum* L.) and garlic chive (*Allium tuberosum* Rottler ex Sprengel). Energy dispersive X-ray spectroscopy (EDS) spectra from FESEM-based experiments revealed the elemental composition and distribution. The results contribute to a better knowledge, characterization, and identification of the *Allium schoenoprasum* L. and *Allium tuberosum* Rottler ex Sprengel species by revealing the basic, primary, secondary, and micronutrient minerals present in the pollen grain (Manjunathagowda et al., 2022a, 2022b). FESEM–EDS techniques aid to identification and characterization to understand drought stress in garlic. The mineral composition of root sections, their profiles as particulate matter elemental diffusion, and their use as specific indicators for the identification and confirmation of known reference samples, as well as the identification of unknown samples, could all be important references for identifying genotypes that are tolerant to drought.

CONCLUSION

Under drought stress conditions, the root structures of garlic genotype DOGR-24 exhibit greater stability compared to genotype DOGR-28 in terms of anatomical alterations. Additionally, using overall assays, it was possible to determine how drought stress treatment affected the morphology of root structures by root anatomical alterations and their profiles. For the purpose of enhancement of breeding programs by identifying genotypes based on the FESEM-EDS profiles root analysis, these thorough insights of inner and external growth descriptions are added advantage for the precise identification of genotypes for drought tolerance.

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