

Interactive Effect of Elevated CO₂ and Moisture Stress on Anatomical Configuration in Brassica Species

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

Article Information

DOI: 10.9734/IJECC/2021/v11i230370

Editor(s):

(1) Dr. Arjun B. Chhetri, Dalhousie University, Canada.

Reviewers:

(1) Irina Ielciu, Iuliu Hatieganu University of Medicine and Pharmacy, Romania.

(2) Michael Jonathan Fernandes Alves, Ireland.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/67871>

Received 17 February 2021

Accepted 25 April 2021

Published 01 May 2021

Original Research Article

ABSTRACT

Free-Air CO₂ Enrichment (FACE) was developed as a means to study the crops response to elevated level of CO₂ under the fully open-air field conditions. In this study, results of FACE experiments are summarized by disusing the root and shoot anatomy. Result indicated that elevated CO₂ significantly altered the root and shoot xylem and phloem characters such as both proto and meta xylem and phloem; vessels, character of root and shoots which are vital for the transpiration regulation, along with leaves photosynthesis as a whole. The coexistence of two ontogenetically different phloem sieve element in *Brassica* plant under elevated CO₂ might have possibility of two different transport functions at the same time. One may be involved in supplying for the structural development (leaf size, stem girth and root volume) and other may cater the need of increased new sinks. Though these parameters were found to decrease under moisture stress condition but these impacts of stress were reduced at higher level of atmospheric CO₂.

Keywords: Elevated CO₂; root; phloem; xylem; moisture stress and vessel.

1. INTRODUCTION

Since the pre-industrial period concentration of atmospheric CO₂ has increased which was

280 ppm before pre-industrial revolution and it has become 391 ppm in 2011 as per IPCC report, [1]. If the current high emission trend continues, CO₂ is predicted to exceed 1,000

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ppm by the year 2100 [2]. Associated climate changes such as increased atmospheric vapour pressure deficit will likely increase water use in agriculture, thereby increasing soil drying leading to more severe regional or global drought and food shortage [2].

Plant has some ability to adapt in higher level of atmospheric CO₂ and evidence indicates there is link between variations in atmospheric CO₂ and plant structural adaptations. Report suggests that elevated CO₂ alters plant makeup by changing some important processes viz. cell division, cell expansion and cell cycling. This alteration in plant at organ level is possibly due to metabolic changes might be due to elevated CO₂ [3]. Climate models forecast a terrestrial air temperature increase of 1.2–4.8 °C by the end of the 21st century [2]. It was reported that increasing CO₂ concentrations in the atmosphere stimulates root growth and enhanced soil exploration [4-5]. Such beneficial effects of elevated CO₂ enhanced the proliferation of roots into resource rich microsities [6] and increased water and nutrient use efficiency [7-8]. Plant's water relations has influenced by the modification of vasculature in stem under elevated CO₂ and has received much attention in climate change research programme Sharma et al. [3]. There was some report that of relation between CO₂ induced modification in xylem anatomy and cavitations of stem of *Helianthus annuus* grown in various level of CO₂ as reported by [9]. According to them diameter of vessel has been shown to enlarge and inadequate carbon availability at low CO₂ which leads to weakened pit membrane of xylem conduit and as a result of this xylem cavitations but at sufficient carbon availability and reduced water demand at high resulted in thicker conduit wall, narrower conduit diameter and robust pit membrane. There was variation within the species in alteration in anatomy of vessel elements due to elevated CO₂ [10]. Similarly increased vessel diameter has been reported in beech [11] and aspen by [12] under elevated CO₂ condition. They had opined that there was a chance of increase in vessel diameter might be due to development of more tension resulting cavitation of xylem and embolism of vessel. Plant growth responses to environmental changes may be

linked to xylem anatomical adjustments [13].

Species specific response of xylem to high atmospheric level of CO₂ was also reported by [14] in the stem of some leguminous species (*Vicia hybrida*, *Trifolium repens*, *Vicia sativa*) grown under Free air CO₂ enrichment technology. Tracheary elements of *T. repens* at higher CO₂ level reduced lignifications causing CO₂ –enhanced decline of precursor of lignin but no such modification was found in *V. hybrida* and *V. sativa*. Moreover, vessel lumen of both these species tapering because of elevated level of CO₂ indicating a decrease in hydraulic conductance under elevated CO₂. In contrary, vessel lumen of *T. repens* remained unaffected [14]. Secondary growth of stem was also affected due to its effect on activity of fascicular and inter-fascicular cambium under higher level of atmospheric CO₂ [15]. Size and number of constituents of secondary xylem also affected by elevated CO₂. In terrestrial vegetation long-distance, less resistance water transport path, xylem allows quick and extensive movement of water from roots to foliage and is required to maintain gas exchange in leaves under dry environment [16]. Therefore elevated CO₂ may have positive response to alleviation of tress by modifying or altering the xylem physiology. And various studies has indicated that alteration of anatomy of stem pattern at elevated CO₂. Further studies are needed to recognize its ramifications and connection with continuing rise in atmospheric CO₂ content primarily in timing and mechanism of plant alteration to higher level of CO₂ is necessary in more number of species of both tropical and temperate plants. Because anatomical modification which will have significant influence in the biomass production potential or productivity of some crop including *Brassica species* viz. *Brassica juncea* cv. 'RH-30' and *Brassica campestris* cv. 'Pusa Gold' under elevated CO₂ and moisture stress condition. The main aim of this study is to find out the spatial and functional aspects of plant root and shoot anatomy under elevated CO₂ condition and to help provide data base for simulation models model, identification of cultivars and modification of cultivation and nutrient application technologies for future environments.

2. MATERIALS AND METHODS

2.1 Plant Material

Brassica cultivars viz. *Brassica juncea* cv. RH-30 and *Brassica campestris* cv. Pusa Gold were collected and grown for the present investigation.

2.2 Experimental Site and Growth Conditions

The response of both the species to elevated CO₂ was studied using Free Air CO₂ Enrichment Technology (FACE) to simulate the doubling CO₂ concentration at, IARI, New Delhi-12. The crops were grown in the field and inside the Mid Free Air CO₂ enrichment (FACE) facility in 8 m diameter circles. An elevated CO₂ concentration of 550 μmolmol^{-1} was maintained throughout the crop growth period with the help of computer-based PID valves. There was no exogenous supply of CO₂ to the normal air under ambient field condition. Field was prepared by recommended agronomic practices.

2.3 Cultural Practice

Farmyard manure was applied at the rate of 5 tons per hectare at the time of field preparation. The plant spacing, fertilizer application at the rate of 30+30:60:40 kg per hectare of nitrogen, phosphorus and potassium and other cultural practices were followed as reported by Uprety et al. [17]

2.4 Moisture Stress Treatment

Moisture stress treatment was given by restricting irrigation and bringing the soil moisture level between 7 and 10% compared to 22-25% under irrigated condition. All the observations were taken in triplicate for each treatment at Stage-1: vegetative (25 days after sowing), Stage-2: flower bud initiation (45 DAS), Stage- 3: 50% flowering (60 DAS) and Stage-4: post flowering (75DAS).

2.5 Root and Stem Anatomy

To study the stem and root anatomy, 5 mm thick stem and root section were cut and fixed in the fixative solution (FFA) and then dehydrated in series of alcohol dilutions (70 to 100%), treated further with xylene and

embedded in paraffin wax as suggested by Jensen [18]. The xylem and phloem tissues were characterized after staining with safranine. Calibration of microscope was done with the help of stage and ocular micrometer and structural parameters were determined under microscope (100x and 60x magnification) [19].

3. RESULT

3.1 Shoot Anatomy

3.1.1 Vessel characters

Significant increase in different vessel characters were observed with elevated level of CO₂ Fig: 1. Increase over the ambient plant was 24 % (vessel number), 28 % (length), 15 % (breath) and 43 % (area). The more vessel number, length, breath, and area were observed in RH-30.

Moisture stress treatment significantly reduced the vessel characters. The reduction was 39% (in vessel number), 33% (in length), 15% (in breath), and 77% (in area). The stress-induced reduction above parameters in Pusa gold under ambient condition was 38.46% (vessel number), 28.72% (length), 31.48% (breath) and 44.35 % (area) where as elevated condition 20.2% (number), 20.0% (length), 19.08% (breath) and 35.27% (area). The stress-induced reduction in RH-30 under ambient condition was 35.29% (vessel number), 22.99% (length), 27.77% (breath) and 44.35% (area, where as RH-30 15.78% (number), 14.79% (length), 15.72% (breath) and 28.21% (area).

3.1.2 Shoot phloem characters

Significant increase in phloem characters were observed with elevated level of CO₂ Fig 2. The increase over the ambient plant varied from 31 % (number), 36 % (length), 38% (breath) and 82% (area). The more phloem number and length, breath, and large area of phloem were observed in RH-30. Moisture stress treatment significantly reduced the phloem characters. The reduction was 31 % (number), 42 % (length), 42 % (breath), and 96 % (area). The stress induced reduction in Pusa gold under ambient was 28% (phloem number), 33% (length), 40% (breath) and 60% (area) where as elevated was 22% (phloem number), 21% (length) 23% (breath) and 44 % (area). The reduction in RH-30 under ambient was 25%

(phloem number) 30% (length), 37% (breath) and 57% (area) where as in elevated condition was 14% (phloem number), 17% (length), 16% (breath) and 31% (area).

3.2 Shoot Xylem Characters

3.2.1 Metaxylem

The elevated level of CO₂ significantly increased in different meta xylem characters Fig. 3. The increase over the ambient plant was 36 % (meta xylem number) and 34 % (length), 34 % (breath) and 74 % (area).The more meta xylem number, length, breath, and area were observed in RH-30. Moisture stress treatment significantly reduced the meta xylem characters. The reduction was 42 % (number), 38 % (length), 38 % (breath), and 84 % (area). The stress induced reduction in Pusa gold under ambient was 33% (number), 31% (length), 31% (breath) and 53% (area) where as under elevated reduction was 25% (number), 16% (length), 19% (breath) and 35% (area). The stress induced reduction in RH-30 under ambient was 30 % (number), 31 % (length), 31% (breath) and 53%(area) whereas under elevated condition reduction was 18% (number), 16% (length), 14% (breath) and 28.0 % (area).

3.2.2 Shoot proto xylem

Significant increase in proto xylem characters were observed with elevated level of CO₂ Fig.4. The increase varied from 30 % (proto xylem number), 35% (length), 30 % (breath) and 72 % (area). The more proto xylem number, length, breath, and area were observed in RH-30. Moisture stress treatment significantly reduced the proto xylem characters. The reduction was 42%(number), 41%(length), 34%(breath), and 87%(area).The stress induced reduction in Pusa gold under ambient was 36%(number), 33%(length), 33%(breath) and 55%(area) where as in elevated condition reduction was 23%(number), 20% (length),17% (breath) and 34%(area).The reduction in RH-30 under ambient was 33%(number), 25% (length), 25%(breath) and 44%(area) where as reduction in elevated was 16%(number), 16% (length), 13%(breath) and 27(area)

3.2.3 Root anatomy vessel characters

CO₂ enrichment brought about significant increase in vessel characters Fig. 5. The

increase over the ambient plant varied from 29 % (vessel number) and 34 % (length), 33 % (breath) and 74 % (area). The more vessel number, length, breath, and area were observed in root of RH-30. Moisture stress treatment significantly reduced the vessel characters. The reduction was 38 % (number), 39 % (length), 37 % (breath), and 82 % (area). The stress-induced reduction in Pusa gold under ambient was 35 % (number), 35 % (length), 37% (breath) and 59% (area) whereas, elevated was 21(number), 17 % (length), 16 % (breath) and 31% (area). The reduction in RH-30 under ambient was 30% number, 33% length, 33% breath and 55% area where as elevated was 16% (number), 17% (length), 14% (breath) and 28% (area).

3.2.4 Root phloem character

There was significant increase in phloem characters which was observed under elevated level of CO₂ Fig. 6. The increase over the ambient plant was 31% (number), 32 % (length), 31 % (breath) and 73 % (area).The more phloem number, length, breath, and area were observed in root of RH-30. Moisture stress treatment significantly reduced the phloem characters. The reduction was 31%(number), 40%(length), 37%(breath), and 93%(area).The stress induced reduction in Pusa gold under ambient condition was 28% number, 33% (length), 30% (breath) and 53% (area) in whereas reduction in elevated CO₂ condition was 23% (number), 23% (length), 22% (breath) and 44% (area).The stress-induced reduction in RH-30 gold under ambient condition was 25% (number), 26% (length), 27%(breath) and 46% (area) whereas reduction in elevated CO₂ condition was 16%(number), 18%(length), 19% (breath) and 34%(area).

3.2.5 Root xylem characters

CO₂ enrichment significantly brought about the changes in different meta xylem characters (Fig 7) . The increased over the ambient plant was 31%(number) and 35%(length), 32%(breath) and 80%(area).The more metaxylem number, length, breath, and area were observed in root of RH-30. Moisture stress treatment significantly reduced the meta xylem characters. The reduction was 38%(number), 40%(length), 41%(breath), and 92%(area).The stress induced reduction in Pusa

gold under ambient condition 35% (number), 35% (length), 38% (breath) and 80% (area) where as reduction in elevated CO₂ was 20% (number), 19% (length), 19% (breath) and 34% (area). The stress-induced reduction in RH-30 under ambient condition 31% (number), 33% (length), 33% (breath) and 55% (area) where as reduction in elevated CO₂ was 18% (number), 18% (length), 16% (breath) and 31% (area).

CO₂ enrichment significantly brought about the changes in different meta xylem characters. The increase over the ambient plant varied from 32% (number), 38% (length), 35% (breath) and 42% (area). The more proto xylem number, length, breath, and area were observed in root of RH-30. Moisture stress treatment significantly reduced the proto xylem characters. The reduction was 42% (number), 43% (length), 39% (breath), and 44% (area). The stress induced reduction in Pusa gold under ambient condition 40% (number), 35% (length), 34% (breath) and 57% (area) where as reduction in elevated CO₂ was 22% (number), 23% (length), 22% (breath) and 40% (area). The stress induced reduction in RH-30 under ambient condition 35% (number), 31% (length), 30% (breath) and 51% (area) where as reduction in elevated CO₂ was 16% (number), 21% (length), 18% (breath), 36% (area).

4. DISCUSSION

Environmental factors viz. such as temperature and rainfall plays a pivotal role in initiation and development of plant vascular tissue including xylem and phloem, from the vascular cambium [20] even under elevated level of CO₂. Results of present investigation indicated that the elevated CO₂ significantly altered the vessel characters of shoot and root. The vessel numbers, size (length, width and transactional area and pith) were increased due to the CO₂ enrichment. All these characters were adversely affected by moisture stress. Stress effects on these anatomical features were partially reversed due to the elevated CO₂. Brodribb [21] reported that survival and productivity of plants depends upon the xylem hydraulic characteristics. According to him this hydraulic activities are again influenced by the anatomical organization of vessel or tracheid in xylem. Tyree & Zimmermann, [22] also reported that according to Hagen–Poiseuille

law, xylem the water transport efficiency) is related to the vessel number and the fourth power of the vessel diameter. Therefore, elevated CO₂ has greater influence on hydraulic conductivity though xylem property alteration.

Plants has tendency to maintaining the efficiency of water movement and potentiality by regulating the structural components of the water conducting system [23] in any stress condition example CO₂ enrichment was found to enhance the wider vessel lumina [24]. It was apparently seen that elevated CO₂ caused the formation of solid pith under irrigation and partially solid pith under moisture stress. The hollow pith was found in the stem of control plants Fig. 8. Elevated CO₂ depressed the degeneration of parenchyma cells during ontogenic development of tissue. The solid pith was consisted of parenchyma cells. Vessel roundness with diameter, stem cross-sectional of area stem, specific hydraulic conductivity, and embolism decreased under moisture stress condition but these impacts of stress were reduced at higher level of atmospheric CO₂ [16].

Significant alteration in both meta and proto xylem and phloem characters were observed in shoots and roots due to elevated CO₂. It was noted that elevated CO₂ significantly reduced the pith size due to increase in area and number of secondary xylem and phloem (Plate 1). This alteration in xylem and phloem anatomy may increase the stem and root girth. Increased vessel transactional area was an important contributing factor for increasing stem girth. Xylem anatomical structures define key functions for plant survival and performance [23] Plavcová and Jansen, [25] and small changes in xylem anatomical characteristics (e.g. in the lumen diameter of vessels) can considerably improve xylem functioning and ultimately influences yield.

The increase in radial width of xylems (meta+proto) and phloem (meta+proto) increased under elevated CO₂ (Plate 2) indicated that more transfer of water and nutrient from soil to plants resulting higher water status maintained throughout the growth period and increase in phloem size helped to transfer all the solute material from source to

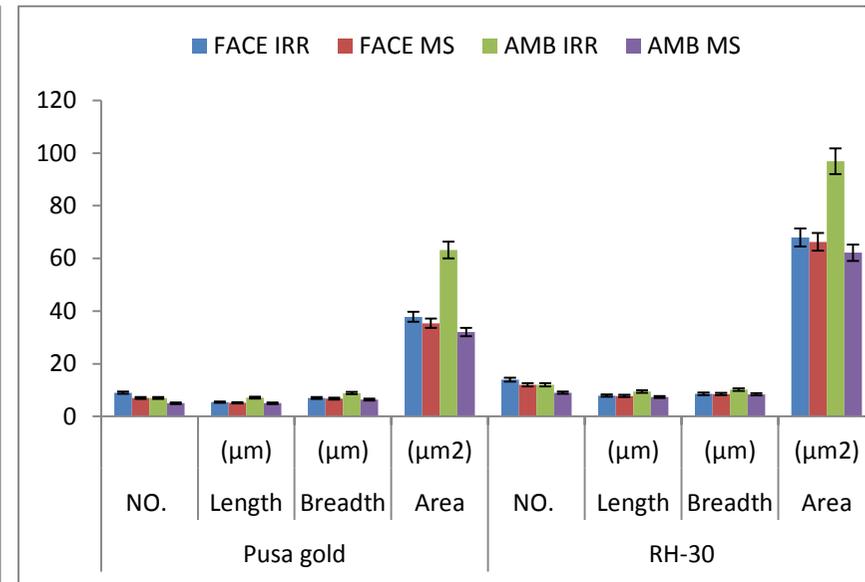
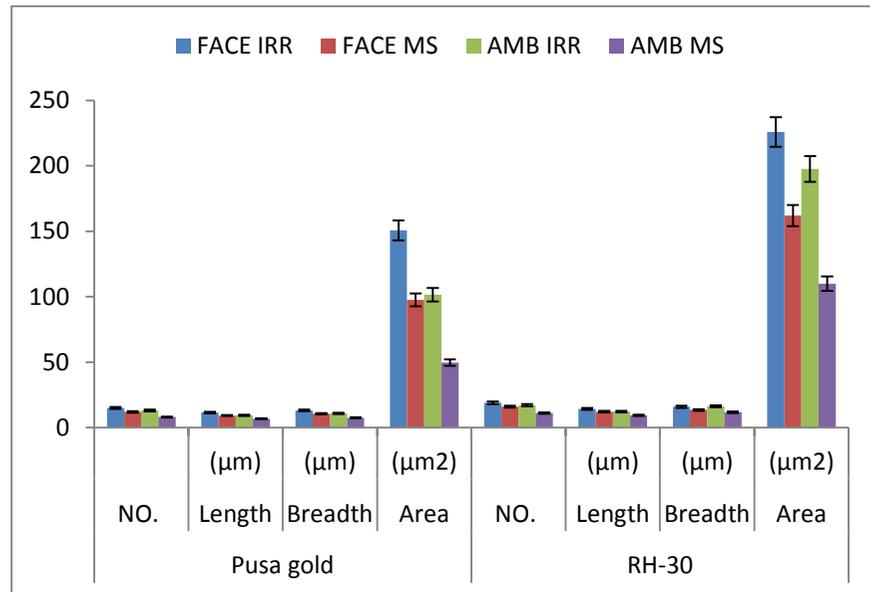


Fig. 1. Inter active effect of CO₂ and moisture stress on vessel characters of shoot

Fig. 2. Inter active effect of CO₂ and moisture stress on phloem characters of shoot

FACE = Free air CO₂ Enrichment ; IRR= Irrigation, MS = Moisture stress , AMB= ambient

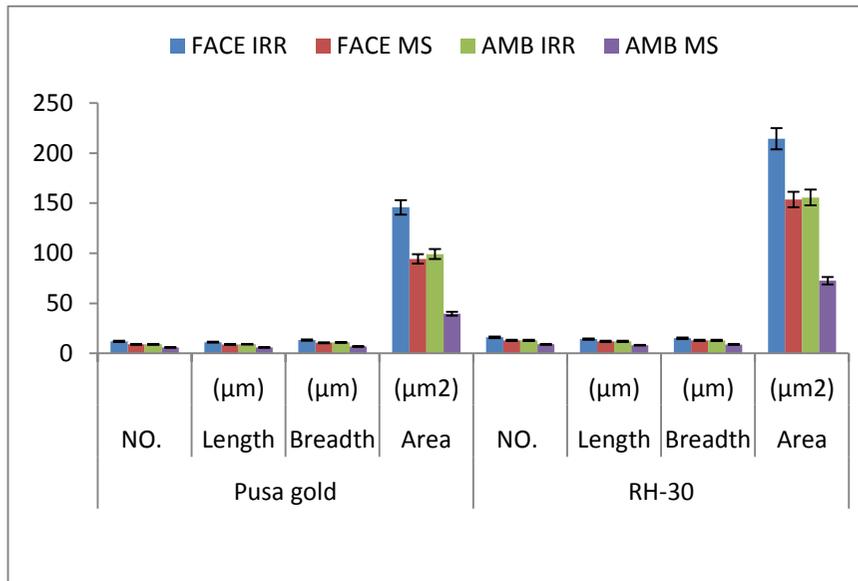


Fig. 3. Inter active effect of CO₂ and moisture stress on meta-xylem characters of shoot

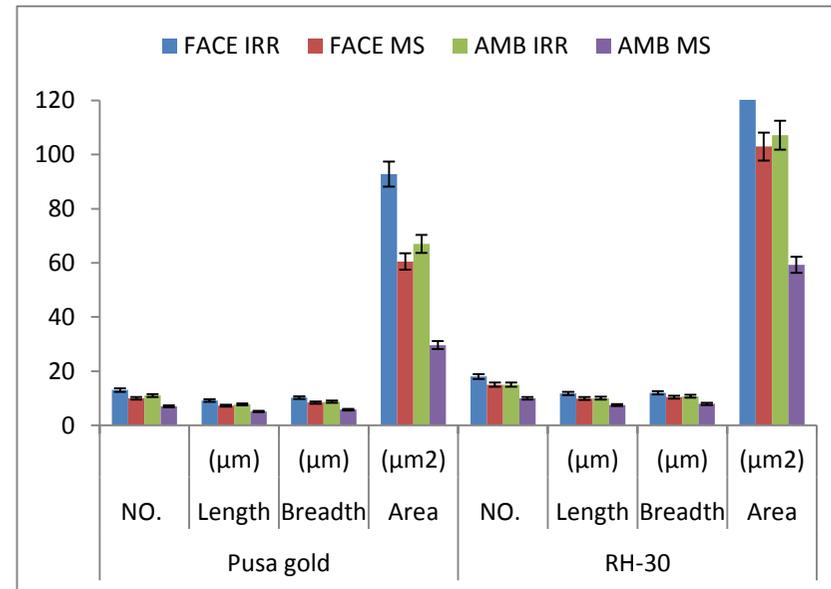


Fig. 4. Interactive effect of CO₂ and moisture stress on proto-xylem characters of shoot

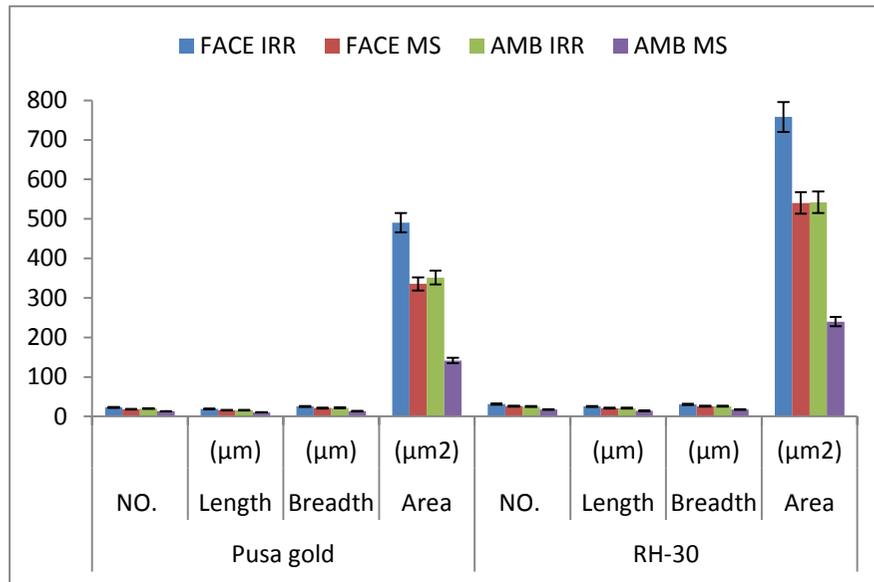


Fig. 5. Inter active effect of CO₂ and moisture stress on vessel characters of root

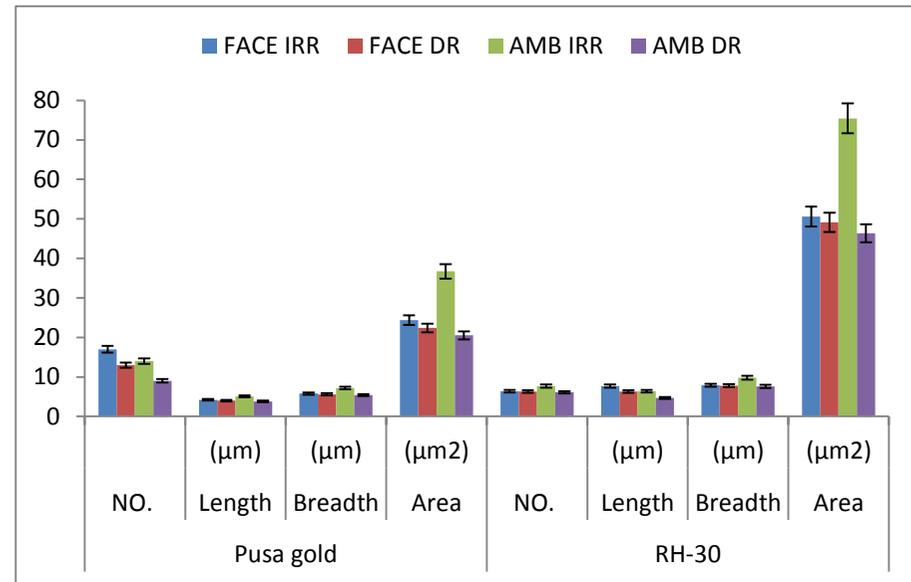


Fig. 6. Inter active effect of CO₂ and moisture stress on phloem characters of root

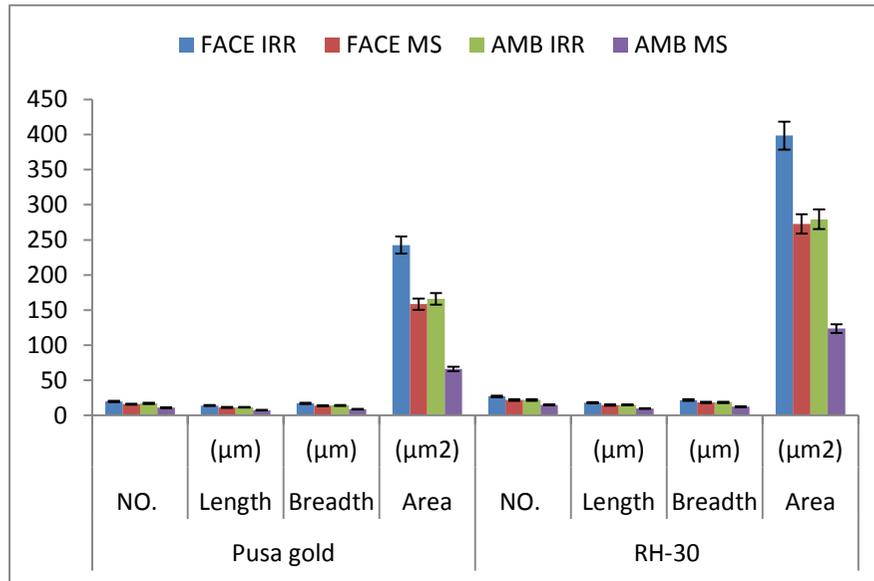


Fig. 7. Inter active effect of CO₂ and moisture stress on meta-xylem characters of root

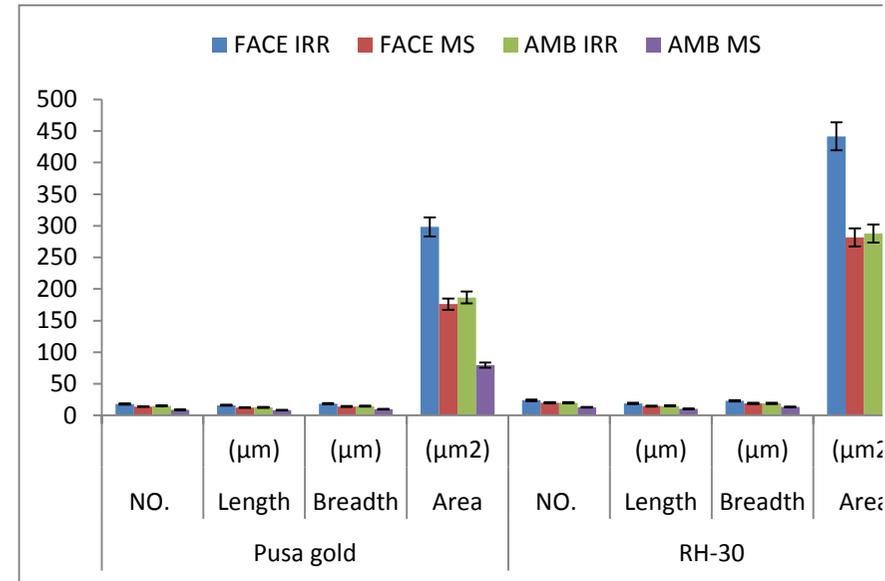


Fig. 8. Inter active effect of CO₂ and moisture stress on proto-xylem characters of root

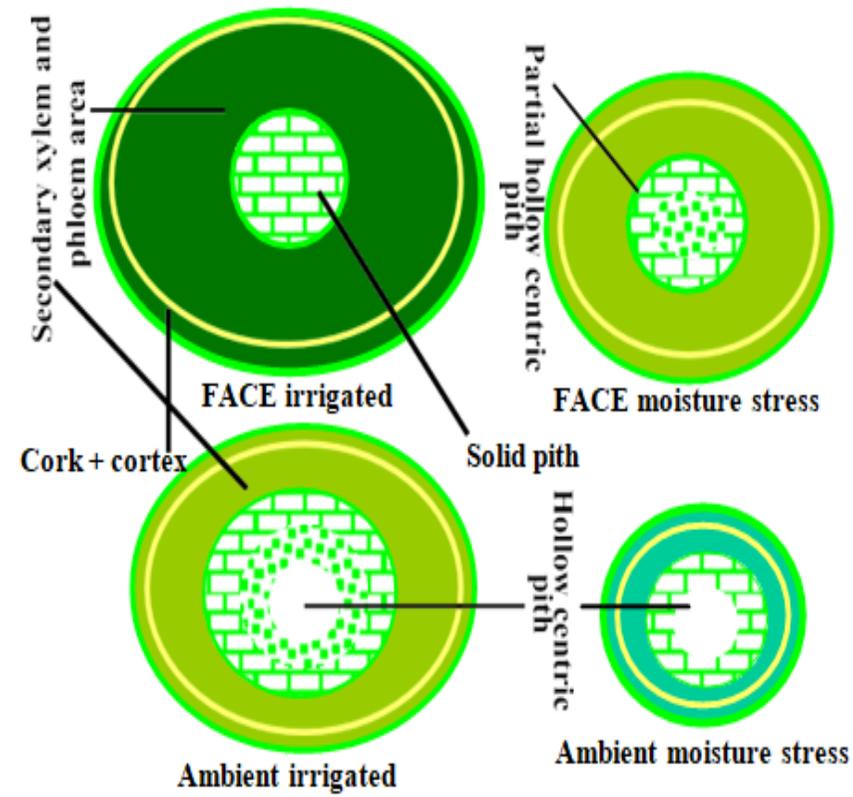


Fig. 9. Diagrammatic presentation of transverse section of main shoots of *Brassica* species

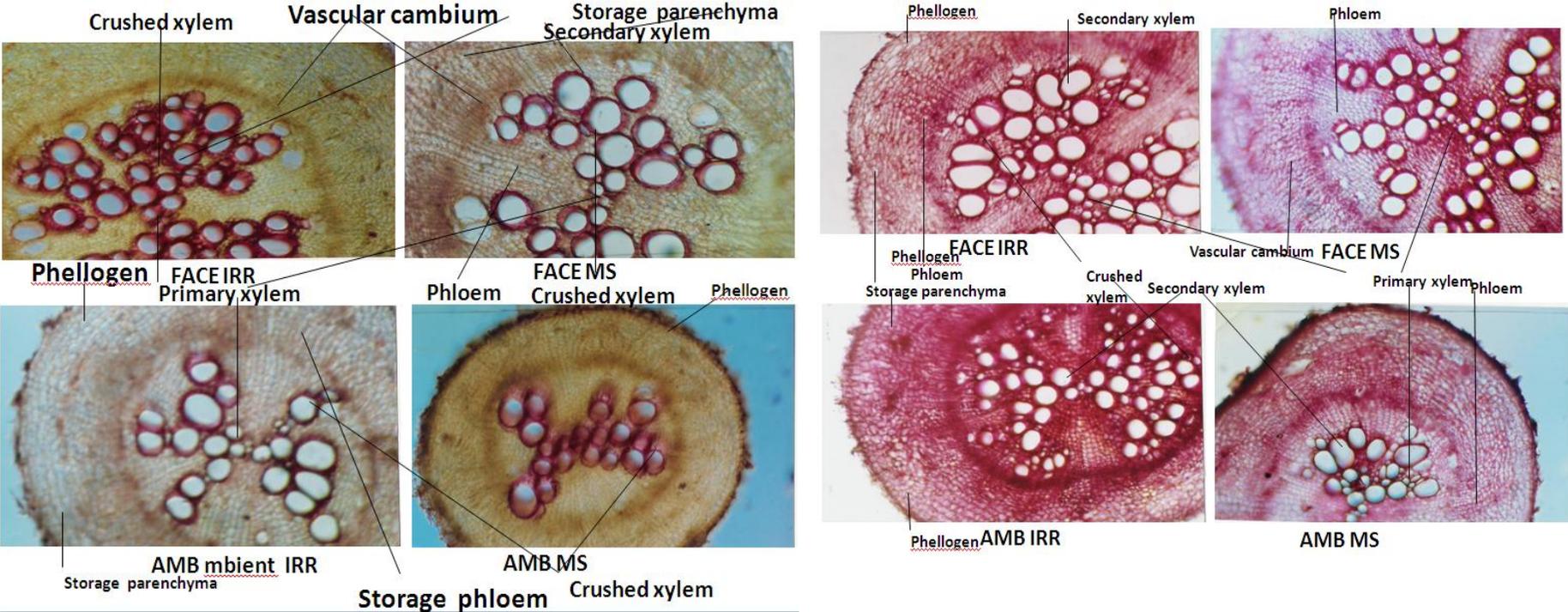


Plate 1. Effect of elevated CO2 and moisture stress on root anatomy of *B. campestris* cv PusaGold and *B. juncea* cv RH-30 (LM 100x) (LM 100x)

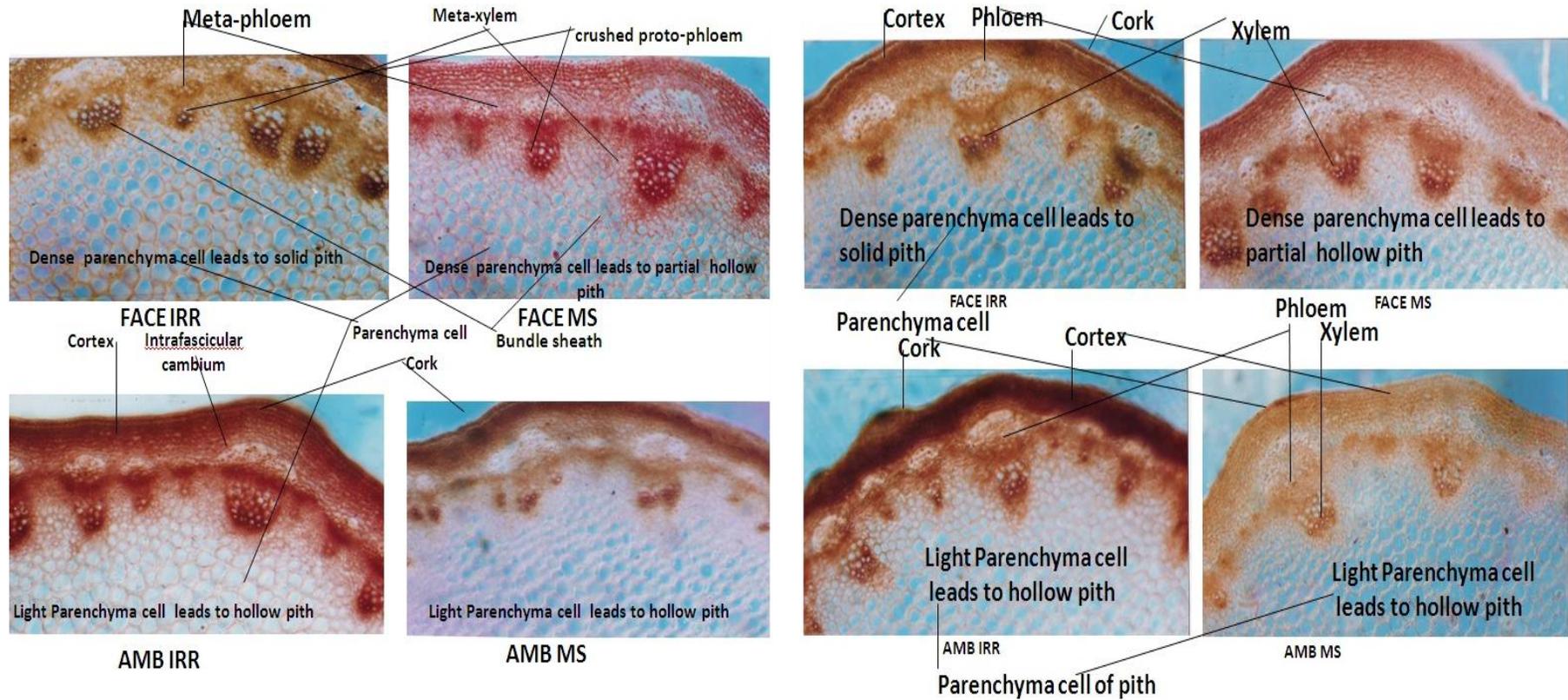


Plate 2. Effect of elevated CO₂ and moisture stress on shoot anatomy of *B. campestris* cv Pusa Gold and *B. juncea* cv RH-30 (LM 100x)(LM 100x)

sink. The proto-phloem sieve element solely imports solute for growth [26]. Meta-phloem functions primarily to transport solute to other developing regions of plant. [27] Wood et al. 1994). Both phloem might have played a greater role in *Brassica* crop as their simultaneous development of vegetative and reproductive growth happened for certain period of time. Therefore, increase in both the phloem size might have helped in the modification for unloading of carbohydrates to sink region following increase in sink size (number of siliqua/plant⁻¹) and sink capacity in *Brassica*. Another important consequence of greater phloem size and number in roots which could channelize basipetal movement of the larger amount of carbohydrate to the roots leading to the development of more number of secondary roots, increased root length and root volume in *Brassica* spp. Therefore we can also hypothesize that the coexistence of two ontogenetically different phloem sieve element in *Brassica* plant under elevated CO₂ indicated the possibility of two different transport functions at the same time. (a) One may be involved in supplying for the structural development (leaf size, stem girth and root volume) and (b) Another may cater the need of increased new sinks. This type of metabolite adjustment through phloem was one of the important areas of the future study related to acclimation crop to higher level of CO₂ elevated CO₂ condition as development of vascular tissue is vital for the transpiration regulation, along with leaves photosynthesis as a whole [20].

5. CONCLUSION

From present study it may concluded that Anatomical strategy developed by the plants under elevated CO₂ to counter drought effect by improving xylem and vessel characters and also showed a significant change in proto-phloem sieve that may conduct solute for structural development and meta-phloem helped in the transfer of photosynthate for inducing new sink development. Improvement in xylem structural organization helped plants to regulate the movement of residual water under stress condition and to maintain greater water status for active physiological process by delaying the onset of moisture stress.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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