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ORIGINAL ARTICLE

# Screening of forty Indian *Amaranthus hypochondriacus* cultivars for tolerance and susceptibility to tropospheric ozone stress

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## Abstract

Tropospheric ozone stress adversely affects crop cultivars growth and productivity variably. The variable response of crop cultivars raised the need for identification of ozone ($O_3$) stress-tolerant cultivars as an adaptive option. In the present study, forty cultivars of *Amaranthus hypochondriacus* were screened for susceptibility and tolerance to ozone stress. The cultivars were exposed to ambient ($AO_3$) and elevated ($EO_3$) ozone levels in free air ozone enrichment (FAOE) facility and monitored for foliar ozone injury symptoms appearance and yield attributes response. Foliar ozone injury symptoms on Amaranthus cultivars were interveinal yellow or black spots. Foliar ozone injury was observed in almost half of the cultivars and the maximum foliar injury (> 75%) was observed in cultivar IC-5527. The maximum yield reduction (> 90%) was observed in cultivars IC-4200 (94.9%) and IC-5569 (91.4%) compared to other cultivars. The results showed that Amaranthus cultivars exhibited variable response towards ozone stress where foliar ozone injury does not always correspond with grain yield reductions. Among the indices, Relative Yield Index (RYI), Stress tolerance (TOL), Abiotic Tolerance Index (ATI), Susceptibility Index (S) and Stress Susceptibility Index (SSSI) were positively correlated with relative yield loss in all the cultivars under ozone stress. With the help of cluster analysis and principal component analysis (PCA), the cultivars were categorized into ozone tolerant, moderately ozone tolerant and ozone susceptible category. The most tolerant cultivars were IC-5527 and IC-1733 which exhibited lower yield losses whereas the most susceptible cultivars were IC-3599 and IC-7924 having high foliar injury and maximum yield losses as compared to other cultivars. The most ozone tolerant cultivars of Amaranthus identified in this study may be recommended for cultivation to farmers in the areas experiencing $EO_3$ during the Amaranthus crop growth period.

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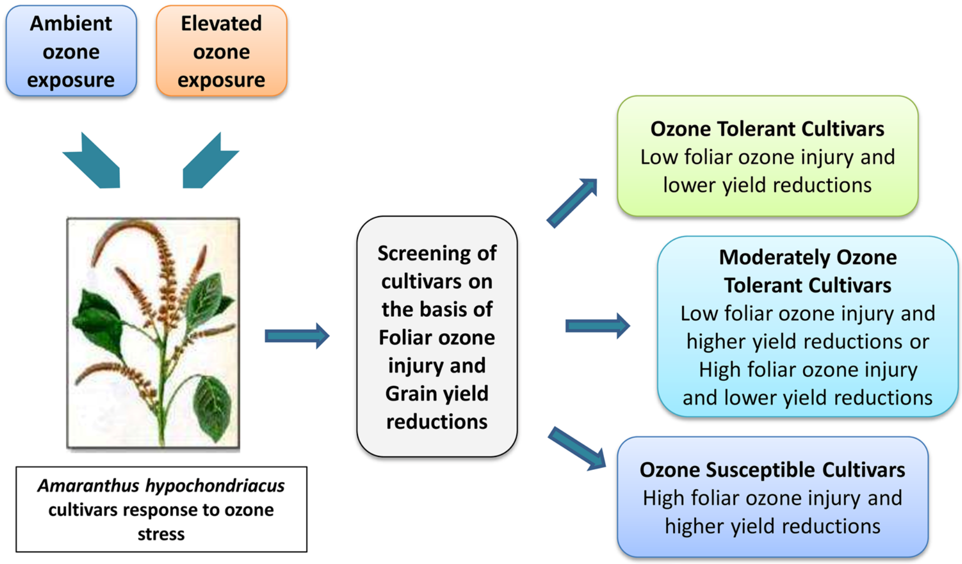
## Graphic abstract




**Keywords** Tropospheric ozone · *Amaranthus hypochondriacus* · Foliar ozone injury · Grain yield · Ozone tolerant and susceptible cultivars

## Introduction

Agriculture plays significant role in socio-economic fabric of India as 18% of India's GDP directly depends on it [49]. Around two third of the working population still rely on agriculture as their principal source of livelihood [39]. It has been well established that elevated level of tropospheric ozone ($O_3$) is phytotoxic and causes negative impact on agricultural crop productivity worldwide [3, 11, 51, 52]. In the upper troposphere, ozone acts as an important greenhouse gas and contribute largely to the oxidation efficiency of atmosphere whereas in lower troposphere (surface level) ozone is more phytotoxic to plants by acting as a strong oxidant [7]. The background ozone levels are rising over mid-latitudes of Northern Hemisphere at a rate of 0.5–2% per year which results in exceedance of the critical levels of ozone in few parts of India [1, 48, 50].Therefore, it is important to explore the options for the sustainability of agricultural crop productivity under increasing surface ozone concentrations over major agricultural regions of India [29]. The identification of ozone tolerant cultivars of various crops is an option that can minimize the yield loss under elevated surface ozone concentrations [31, 40].

The impact of ozone phytotoxicity depends upon the severity and duration of ozone exposure and plant phenotype as well as genotype [16, 56]. Ozone enters plant leaves primarily through the stomata [28, 47, 53]. Once inside the leaf, ozone reacts rapidly with cell components and produces Reactive Oxygen Species (ROS) such as hydrogen peroxide ($H_2O_2$), superoxide radicals ($O^{2-}$), and hydroxyl radicals ($OH^{\bullet}$) [44, 57]. These ROS trigger downstream antioxidant defense response to buffer the lethal effects of accumulated ROS [36]. On the other hand, when this antioxidant system doesn't get activated or ROS accumulation exceeds the antioxidant capacity, plants experience the damage caused by ozone and exhibit susceptibility towards ozone in the form of foliar injury symptoms and yield loss [20, 45, 46]. Several studies have identified distinct response of crops to acute [high concentration of ozone of > 80 ppb for a short period (hours) of time] and chronic ozone exposure [lower concentration of ozone exposure of < 40 ppb for a longer period of time (months to years)] [2, 9, 23, 27]. Frei et al. (2008) detected significant genotypic variation in rice in tolerance to ozone [17]. Feng et al. (2011) also demonstrated the ozone phytotoxicity in wheat to be genotype dependent [13]. Picchi et al. [38] and Sawada and Kohno [43] have studied the effect of ozone stress on wheat and rice cultivars, respectively and reported that there were few cultivars in which grain yields were high but they exhibited least foliar injury symptoms. These studies establishes that different crops as well as crop cultivars exhibit differential foliar injury symptoms and yield reductions under increased ozone concentrations [6, 42].

Grain Amaranthus (*Amaranthus hypochondriacus*) is a pseudocereal characterized by high nutraceutical value. It is herbaceous C4 crop grown in 17 states of India for both grain and greens. The grains are gluten free and highly nutritious with high content of lysine, arginine and histidine [37]. Also they have high functional value ranging from medicine and cosmetics to biodiesel and economic benefits [4]. Previous literature establishes that it possess high resistance towards drought and salinity [22, 35] which makes it a better adaptation option for climate change and crop diversification [10, 54]. So far no study has been reported on Amaranthus crop response to ozone stress as an important abiotic stress for crops.

Hence, the present study was undertaken to assess Amaranthus cultivars response to tropospheric ozone stress and to identify ozone stress-tolerant cultivars for mitigating the ozone stress impact on Amaranthus crop productivity.

## Materials and methods

### Experimental site and crop management

The present experiment was conducted at Indian Agricultural Research Institute, New Delhi, India (28° 35′ N latitude and 77° 12′ longitudes) between October 2018 and February 2019. Seeds of forty accessions of grain Amaranthus (*Amaranthus hypochondriacus*), specifically suitable to be grown in North and North-Western regions of India, were obtained from National Bureau of Plant Genetic Resources (NBPGR), New Delhi. Seeds of all forty accessions were tested in laboratory for viability and then sown on 12th October 2018. Before sowing the field was prepared and recommended basal dose of fertilizers (60 kg $ha^{-1}$ N as urea, 40 kg $ha^{-1}$ P as single superphosphate, and 40 kg $ha^{-1}$ K as muriate of potash, respectively) were applied. A full dose of phosphorus and potassium were given at the time of sowing while nitrogen was given in two split doses at the time of sowing and at 45 days after sowing (DAS), respectively. The seeds were sprinkled in rows with row to row spacing of 90 cm. The surface was then covered with straws, grasses and shredded leaves. After the four leaf stage, the plants were thinned to maintain plant to plant distance of 20 cm. Manual weeding was done three times and recommended cultivation practices were followed during the crop growth period.

### Experimental setup

The experiment was laid down in randomized block design (RBD). The forty cultivars were grown in eight replicates and were exposed to two ozone treatments. In treatment one, cultivars were exposed to ambient ozone concentration ($AO_3$) while in treatment two, cultivars were exposed to elevated ozone concentration ($EO_3$) in Free Air Ozone Enrichment (FAOE) ring (6 mt Diameter). In FAOE ring ozone was delivered using ozone generator (Systrocom Instruments Ltd, India) and ozone sensors (Tongdy Sensing Technology Corporation) were used for real time detection and monitoring of ambient ozone levels. The $EO_3$ was 30 ± 10 ppb above the ambient ozone levels and the cultivars were exposed to both treatments from the seedling stage until plant maturity.

### Meteorological parameters

During the experimental period, meteorological parameters such as minimum and maximum temperature, rainfall, relative humidity, sunshine hours and average wind speed were recorded. The meteorological data was provided by the automatic weather station installed by Indian Meteorological Department (IMD) and Division of Agricultural Physics of Indian Agricultural Research Institute, New Delhi, India. The maximum and minimum temperature ranged between 16.5–35.6 °C and 0.5–20.6 °C, respectively. The total rainfall was maximum in February (2.6 cm). Relative humidity was maximum in February (85.0%) and minimum in the month of October (68.8%). Mean sunshine hours were maximum in October (6.8 h) and minimum in February (3.6 h). Meteorological data monitored during the experimental period is provided in Table 1.

### Ozone monitoring and AOT40

The ozone levels at the experimental site were monitored from 9:00 h to 17:00 h for both the treatments throughout the crop growth period. For each treatment, the accumulated ozone exposure index (AOT40) was accounted for each cultivar with respect to foliar ozone injury appearance as well as yield loss. AOT40 (accumulated exposure over a threshold of 40 ppb) was calculated as accumulated value

**Table 1** Meteorological data during the study period from October 2018 to February 2019

| Month, year | Mean temp (°C) | | Relative humidity (%) | Rainfall (cm) | Sunshine (h) | Average wind speed (Kmph) |
|---|---|---|---|---|---|---|
| | $T_{max}$ | $T_{min}$ | | | | |
| October, 2018 | 32.6 | 14.9 | 68.8 | 0.0 | 6.8 | 2.0 |
| November, 2018 | 27.4 | 8.3 | 73.5 | 0.1 | 4.7 | 2.7 |
| December, 2018 | 21.8 | 5.0 | 79.0 | 0.0 | 4.4 | 1.9 |
| January, 2019 | 20.0 | 6.4 | 82.2 | 3.8 | 1.7 | **3.3** |
| February, 2019 | 21.3 | 10.0 | 85.0 | 2.6 | 3.6 | 4.6 |

of ozone concentration exceeding 40 ppb per hour for the entire exposure period [12]:

$$\text{AOT40 (ppb.h)} = \sum_{i=1}^{n} [Co_3 - 40]i$$

where $Co_3$ is hourly ozone concentration in ppb and $n$ is number of hours in which $Co_3 > 40$ ppb during the crop growth period.

The mean ambient ozone concentration range at the experimental site was 19.6—35.0 ppb, recorded minimum in the month of December and January and maximum in October. For the entire crop growth period, the mean ozone concentration was recorded as 23.6 ppb though hourly mean ambient ozone concentration exceeded the threshold value of 40 ppb and the AOT40 value ranged from 162.9 ppb.h for ambient ozone concentration to 1811.8 ppb.h for elevated ozone concentration during the exposure period (Fig. 1).

## Foliar ozone injury assessment

Each fully expanded mature leaf of all cultivars with all replicates was examined regularly at an interval of 10 days for visible foliar ozone injury symptoms and data was collected on the appearance time, type and severity of injury. The total number of injured leaves per plant was recorded and the type of injury is identified as flecking, chlorosis, bronzing or necrosis. On the basis of severity of injury symptoms the percentage injury for each injured leaf was calculated and an average percentage injury for each plant was determined. Using the Horsfall and Barratt scale (Table 2) [21, 33, 34] the average ozone injury index was assigned to each cultivar of Amaranthus.

## Morphology

Three plants within each cultivar under each treatment were tagged randomly and analyzed for foliage color, shoot length, inflorescence length, inflorescence color, and number of branches.

**Table 2** Horsfall and Barratt scale for assessing foliar injury

| % injury | Index | Injury rating |
|---|---|---|
| 0 | 0 | No injury |
| 1–6 | 1 | Slight |
| 7–25 | 2 | Moderate |
| 26–50 | 3 | Moderately severe |
| 51–75 | 4 | Severe |
| > 75 | 5 | Very severe |

## Yield attributes

At maturity stage (100–110 days), plants were harvested to record inflorescence weight plant$^{-1}$ and grain weight plant$^{-1}$ (unhusked) of three randomly selected plants within each cultivar under each treatment. Susceptibility and tolerance indices (Table 3) were calculated by using grain yield data for each cultivar under both treatments [30, 32]. On the basis of tolerance and susceptibility indices values, ranks were assigned to each of the index (Supplementary Table 2 and 3). Subsequently the mean rank and standard deviation was calculated and final rank sum (Mean rank + S.D.) was derived. Final rank sum value was used to categorize cultivars into ozone tolerant, moderately tolerant and ozone susceptible category.

## Statistical analysis

The data of forty Amaranthus cultivars with three replicates each was subjected to various statistical tests using XLSTAT (ver. 2014) on MS EXCEL 2007 (Microsoft) software and SPSS software (SPSS Inc., ver. 21.0). The normal distribution of the data within each treatment group was verified by the Shapiro–Wilk W test. The assumption of the homogeneity of the variances was verified for each parameter by Levene's test. Two-way ANOVA was used to test the level of significance of individual effects of treatment on various parameters for each of the test cultivars. Also cluster analysis was applied on the data obtained for

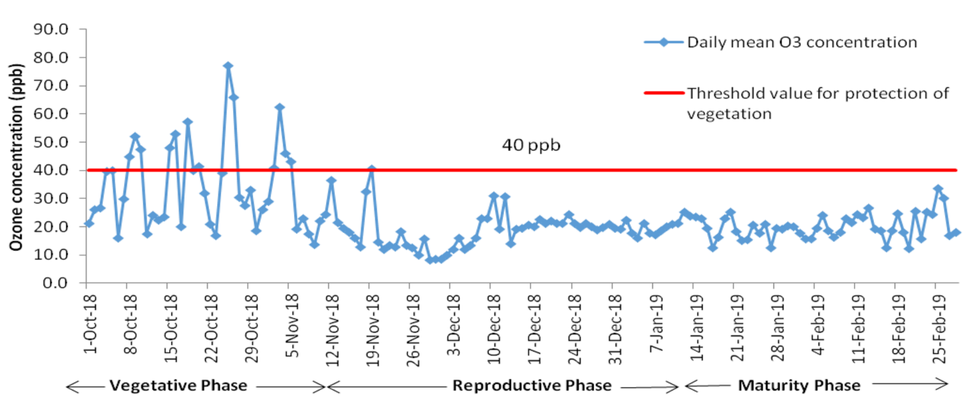



**Fig. 1** Variations in daily mean ozone concentrations (ppb) during crop growth period of Amaranthus with episodes of exceedance of ozone levels above the threshold value set for the protection of vegetation (40 ppb)

**Table 3** Susceptibility and tolerance indices

| Index class | Stress index | Formula | References |
|---|---|---|---|
| Tolerance indices | Yield index (YI) | $YI = \frac{(Yi)s}{(Y)ns}$ | [19, 25] |
| | Yield stability index (YSI) | $YSI = \frac{(Yi)s}{(Yi)ns}$ | [8] |
| | Relative stress index (RSI) | $RSI = \frac{(Yi)s}{(Yi)ns} / \frac{Ys}{Yns}$ | [14] |
| | Relative efficiency (RE) | $RE = \frac{(Yi)s}{Ys} X \frac{(Yi)ns}{Yns}$ | [14] |
| | Stress tolerance index (STI) | $STI = \frac{[(Yi)nsX(Yi)s]}{Yns^2}$ | [14] |
| | Resistance index (RI) | $RI = \frac{(Yi)sX\left(\frac{(Yi)s}{(Yi)ns}\right)}{Ys}$ | [25] |
| Susceptibility indices | Relative yield decrease (RYD) | $RYD = 100 - \left(\frac{(Yi)s}{(Yi)ns} X100\right)$ | [15] |
| | Schneider's stress severity index (SSSI) | $SSSI = \left(1 - \frac{(Yi)s}{(Yi)ns}\right) - \left(1 - \frac{Ys}{Yns}\right)$ | [15] |
| | Stress susceptibility percentage index (SSPI) | $SSPI = \frac{(Yi)ns-(Yi)s}{2(Y)ns} X100$ | [41] |
| | Stress tolerance (TOL) | $TOL = (Yi)ns - (Yi)s$ | [41] |
| | Abiotic stress tolerance index (ATI) | $ATI = \frac{(Yi)ns-(Yi)s}{\frac{Yns}{Ys}} X\sqrt{(Yi)nsX(Yi)s}$ | [24] |
| | Susceptibility index (S) | $S = (Yi)ns - (Yi)s/(Yi)ns$ | [24] |

(Y*i*)*s* and (Y*i*)*ns* denotes the yield of the *i*th genotype and Y*s* and Y*ns* represent the yields of all genotypes under stress and non-stress conditions, respectively

morphological parameters and foliar injury for grouping the cultivars according to their response to ozone stress [26, 55]. Squared Euclidean distance and complete linkage were used for dissimilarity measure and agglomeration. Principal component analysis was conducted on the yield indices and the cultivars were ordered for their ozone susceptibility and tolerance accordingly.

## Results

### Foliar ozone injury assessment

Visible foliar ozone injury was observed in the form of interveinal chlorotic brown and black spots on the adaxial surface of leaf (Fig. 2). Foliar injury was first observed in cultivar IC-5527 at 30 days after sowing (DAS) and in IC-1493 at 33 DAS. At 35 DAS the injury starts appearing in IC-4202, IC-7923 and IC-7031. Initially symptoms appear in the form of small chlorotic spots but later on prominent interveinal chlorosis was observed. Ozone damage was also observed in IC-5569, IC-7922, and IC-7930 at 45 DAS whereas IC-5576 showed injury symptoms at 50 DAS (Fig. 3).

The cultivars showing injury symptoms later than 50 DAS were IC-3599, IC-4203, IC-4207, IC-5575, IC-5621, IC-7437, IC-7836, IC-7916, IC-7924, IC-7926 and IC-7928. No injury symptoms were observed in the plants grown in ambient ozone concentrations (Supplementary Table 6).

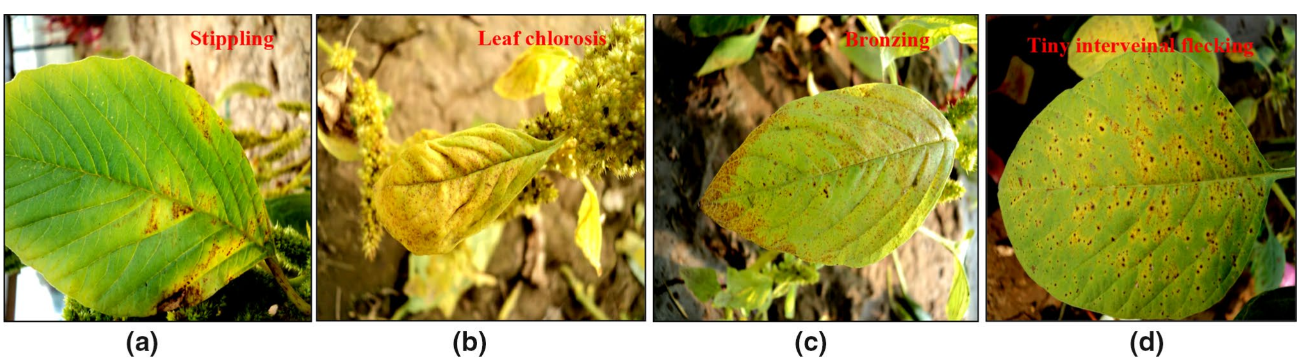



**Fig. 2** Foliar ozone injury of different extent developed on the leaves of Amaranthus cultivars exposed to elevated ozone concentration in the form of interveinal small brown spots and flecks: **a** IC-5576 at 75 DAS, **b** IC-7924 at 68 DAS, **c** IC-4202 at 68 DAS, **d** IC-5527 at 55 DAS

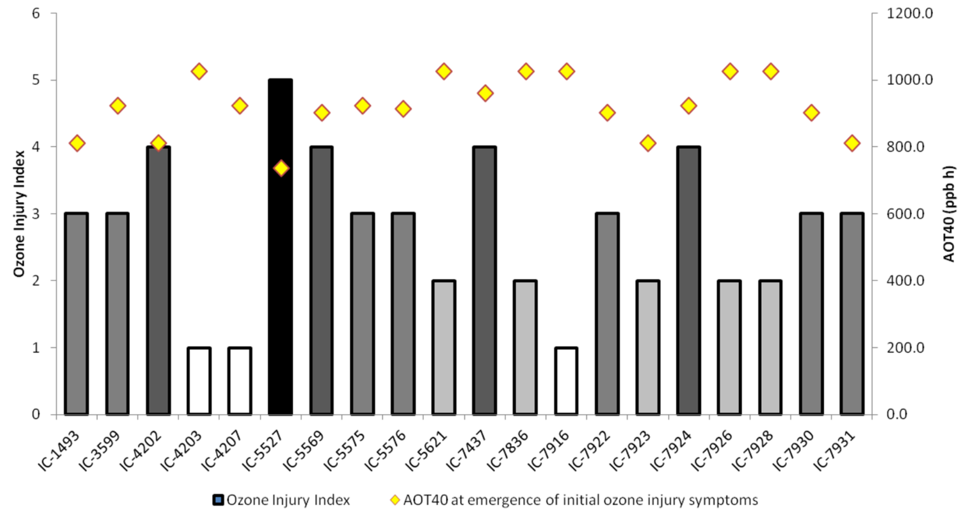



**Fig. 3** Ozone injury index of Amaranthus cultivars exhibiting foliar ozone injury and AOT40 at which initial foliar ozone injury symptoms emerged. The bars indicate the ozone injury index with color codes depicting the severity of the foliar injury: ☐—slight injury, ☐—slightly moderate injury, ☐—moderate injury, ☐—severe injury, ■—very severe injury

## Morphology

The leaf foliage color ranged from light green to reddish green color. The color of the inflorescence ranged from green to pink with the exception of IC-1733 having golden-brown inflorescence. The highest number of branches with longest shoot length was found in IC-3599 whereas longest inflorescence was present in IC-1493 (Supplementary Table 1).

## Cluster analysis

Based on the response of the forty cultivars to ozone stress in terms of visible foliar ozone injury and morphological attributes, agglomeration hierarchical clustering was performed and the relevant dendrogram was created (Fig. 4). The analysis grouped the cultivars into three main clusters: the first cluster (Cluster 1) consisted of cultivars with no ozone injury and intermediate plant maturity (maturity

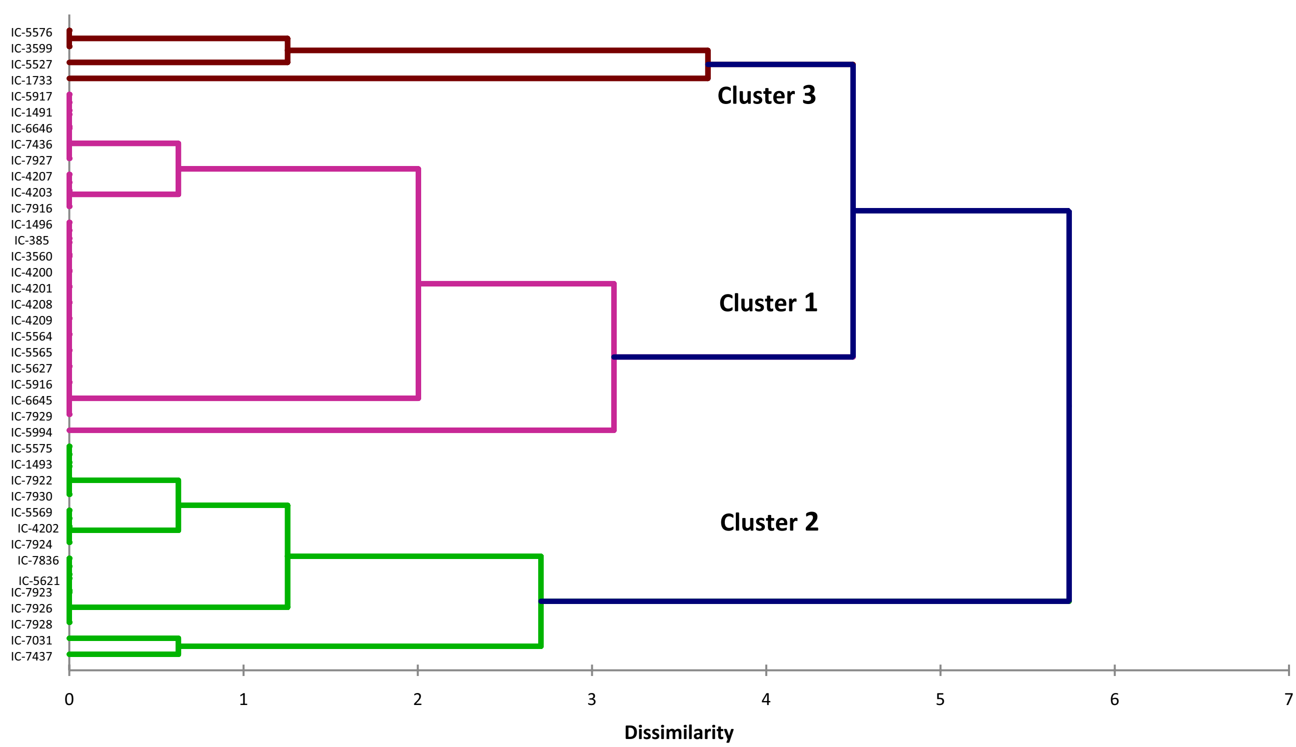



**Fig. 4** Dendrogram grouping of forty *A. hypochondriacus* cultivars based on leaf color, ozone injury index and maturity period. Squared Euclidean distance, Complete linkage, Dissimilarity measure, Agglomeration

period between 100–110 days) and represents ozone tolerant cultivars; the second cluster (Cluster 2) consisted of cultivars with early maturity (90–100 days) showing varying degree of ozone injury and represents ozone susceptible cultivars; and third cluster (Cluster 3) consisted of the cultivars with late maturity (110–120 days) and ozone injury symptoms except IC-1733 which had late maturity without any ozone symptoms and represents moderately tolerant cultivars (Table 4).

### Yield attributes

Grain yield loss was observed in all cultivars of Amaranthus except IC-5627 when exposed to ozone stress. The percent reduction in grain yield was highest in IC-4200 and IC-5569 being 94.9% and 91.4% respectively. This was followed by IC-7924 (89.9%), IC-3599 (89.7%), and IC-4202 (88.7%). The lowest reduction in grain yield was observed in IC-5527 (7.8%) followed by IC-1491 (20.1%), IC-1493 (23.3%) and IC-385 (24.8%). In order to determine the most ozone tolerant and susceptible cultivars accurately, key tolerance indices and susceptibility indices based on grain yield under ozone stress and non stress conditions were considered. A rank was allotted to each index as whole number provides an easier interpretation than decimal values allocated to the original indices values. In order to get an average rank value, a mean value of ranks of stress tolerance indices and stress susceptibility indices along with the standard deviation was calculated and a final Rank Sum (Mean rank + S.D.) was calculated.

In terms of stress indices, the ozone tolerant cultivars were identified having highest rank in tolerance indices whereas ozone susceptible cultivars were those with highest rank in susceptibility indices. The first rank in yield index (YI) was observed for IC-5527 followed by IC-7836 (Rank 2) and IC-5576 (Rank 3) whereas the last rank was for IC-4200 (Rank 40) followed by IC-5569 (Rank 39) and IC-7916 (Rank 38) (Supplementary Table 4).

The best rank for yield stability index (YSI) and relative stress index (RSI) was recorded in IC-5627 (Rank 1), IC-5527 (Rank 2) and IC-1491 (Rank 3) whereas for Relative efficiency (RE) and Stress tolerance index (STI) the best ranks were given to IC-5527 (Rank 1), IC-4201 (Rank 2)) and IC-7836 (Rank 3). In the case of susceptibility indices, for Relative yield decrease (RYD) and Stress severity index (SSI), first rank was recorded for IC-4200 followed by IC-5569 (Rank 2) and IC-7924 (Rank 3). Similarly Abiotic tolerance index (ATI) identified IC-4201, IC-3599 and IC-4207 as susceptible cultivars (Supplementary Table 5). The overall rank sum of cultivars based on mean rank and standard deviation of individual rankings of different indices was calculated with highest value getting the first rank. In terms of rank sum, present study identified IC-5527, IC-1733, IC-385 and IC-7436 as most ozone tolerant cultivars and IC-3599, IC-7924, IC-7437, IC-4202 and IC-7930 as most ozone susceptible cultivars.

The correlation matrix between the relative yield loss (RYL), tolerance indices and susceptibility indices exhibited that most of the indices were significantly and positively correlated with relative yield loss (Supplementary Table 7). The positive correlations of tolerant indices indicate that these indices can be effectively used with other selection parameters for the screening and identification of ozone tolerant and sensitive cultivars. Since the measured and analyzed yield parameters were correlated significantly, the Principal Component Analysis (PCA) was conducted. PCA concentrated more variability in first two principal components (Table 5). The Eigen value of PC1 and PC2 were more than unity and for other PC's it reduced to less than unity which indicates that weight values of PC1 and PC2 are reliable whereas other principal components were not considered. Total variance explained with PC1 and PC2 was 68.86% and 27.19%, respectively. Therefore PC1 and PC2 would be considered as the major axes for cultivar ordinations. Indices such as Yield index (YI), Yield stability index (YSI), Relative stress index (RSI), Relative efficiency (RE), Stress

**Table 4** Categories of Amaranthus cultivars based on cluster analysis

| Cluster | Leaf color | Ozone injury index | Maturity period | Category |
|---|---|---|---|---|
| 1 | Reddish green | 0 | 100–110 | Ozone tolerant |
| 2 | Green | 3–5 | 90–100 | Ozone susceptible |
| 3 | Green/reddish green | 1–3 | 110–120 | Moderately ozone tolerant |

**Table 5** The eigen values and percent variability after principal components analysis of yield indices

| | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 |
|---|---|---|---|---|---|---|
| Eigen value | 8.264 | 3.263 | 0.375 | 0.077 | 0.016 | 0.005 |
| Variability (%) | 68.866 | 27.190 | 3.127 | 0.643 | 0.130 | 0.045 |
| Cumulative % | 68.866 | 96.055 | 99.182 | 99.825 | 99.955 | 100.000 |

tolerance index (STI) and Resistance index (RI) are tolerance indices and have high loading on PC1. On the other hand, susceptibility indices such as Stress tolerance (TOL), Relative yield decrease, Stress severity index (SSSI), Abiotic tolerance index (ATI), Stress susceptibility percentage index (SSPI) and Susceptibility index (S) have high loading on PC2. Therefore cultivars with higher component scores on PC1 are tolerant cultivars and expected to perform better under ozone stress condition whereas cultivars with high component scores on PC2 are expected to perform poorly under ozone stress and are categorized as ozone susceptible cultivars.

A biplot is derived from principal component analysis to compare and categorise the cultivars into ozone tolerant, moderately tolerant and ozone susceptible cultivars (Fig. 5). The biplot of PC1 and PC2 indicated that cultivars with higher component scores of PC1 with higher values of tolerance indices and lower scores of PC2 were highly tolerant to ozone stress. Similarly, cultivars with higher component scores of PC2 with higher values of susceptibility indices were sensitive to ozone stress. Therefore it is concluded that the cultivars on the extreme right of the biplot were tolerant to ozone stress whereas those on extreme left were susceptible to ozone and the cultivars lying in the middle of the plot were categorized as moderately tolerant/susceptible cultivars.

## Discussion

The air quality monitoring data at the experimental site indicated that ambient ozone was high during 9.30 am to 4.30 pm. Therefore elevated levels of ozone ($AO_3 + 30$ ppb) were supplied for 8 h per day from 10.00 am to 5.00 pm throughout the growing period of crop. The $AO_3$ levels went above 40 ppb threshold during the vegetative period but subsequently lowered in reproductive and maturity phase. The lower mean $AO_3$ levels were attributed to the lower temperature and shorter sunshine hours in the month of December and January.

The first instance of foliar injury was observed at the late vegetative phase in the $EO_3$ treatment but no injury was observed in the $AO_3$ treatment which suggests that the observed foliar injury was strictly due to the stress caused by elevated levels of ozone. Since rate of increase of background ozone levels was very slow, the cultivars slowly developed resistance to exceedance events of $AO_3$ levels which helped them to perform without showing any adverse effects. But the sudden exposure to ozone concentrations above the ambient levels affected the plants which lead to foliar ozone injury and grain yield reductions. The foliar injury further aggravated with the exposure period of $EO_3$ which indicated the dependence of foliar injury on the dose of ozone exposure. The AOT40 value of $AO_3$ was calculated to be 162.9 ppb.h which was quite low in comparison to AOT40 of $EO_3$ i.e. 1811.8 ppb.h and also not sufficient enough to induce foliar injury.

Three clusters of cultivars were observed on the basis of foliar injury, foliage color and plant maturity. The

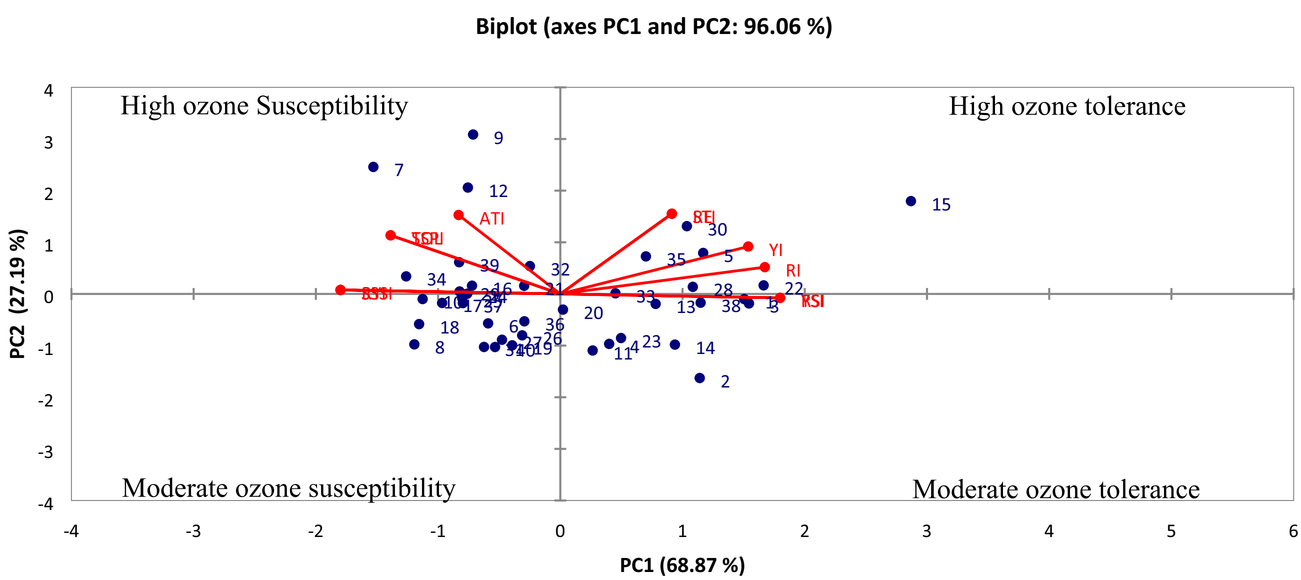



**Fig. 5** Principal component analysis biplot of *A. hypochondriacus* cultivars based on stress tolerance and susceptibility indices

results indicated the occurrence of high foliar injury on plants with early maturity and green foliage color whereas plants with late maturity and reddish green foliage color exhibited very low foliar injury. These observations suggest that there may be other factors influencing the ozone stress tolerance in Amaranthus cultivars which need to be investigated.

In the present study, exposure of *Amaranthus hypochondriacus* cultivars to ozone showed that the cultivars varied in response to ozone stress with respect to foliar injury and grain yield. There were some cultivars which exhibited severe foliar injury but very less reduction in grain yield and also there were some cultivars which exhibited susceptibility to ozone stress in terms of grain yield reduction but foliar injury symptoms were not expressed. The results are well in agreement with earlier studies on rice which suggests the variable response of rice cultivars to ozone stress in terms of foliar injury and yield loss [42]. Frei M. (2015) also showed genotypic variations in rice in response to the ozone stress [18]. The highest and earliest ozone injury was observed in cultivar IC-5527 but reduction in grain yield was only 7.8% which was lowest among all the cultivars. Similarly, IC-4200 exhibited highest reduction in grain yield but exhibited no symptoms of ozone injury. Thus cultivars response to ozone evaluated by extent of visible foliar injury does not always coincide with the reductions in grain yield.

Due to these exceptions, the identification of ozone tolerant and susceptible cultivars on the basis of single criterion of visible injury may be contradictory. In order to determine the most ozone tolerant and susceptible cultivars accurately, key tolerance indices and susceptibility indices based on grain yield under ozone stress and non stress conditions were considered [5]. Amaranthus cultivars IC-5527 and IC-7836 exhibited best rank sum for tolerance indices and identified as most ozone tolerant cultivars while IC-3599 and IC-7924 were identified as most ozone susceptible cultivars due to their best rank for susceptibility indices. These results were further supported by pearson correlation studies and principal component analysis.

PCA showed that cultivars based on the indices tend to group into four categories viz. ozone tolerant, moderately tolerant, moderately susceptible and ozone susceptible cultivars. The first category had higher values of tolerance indices and represents the cultivars suitable to be grown under high ozone concentrations (Tolerant group). The second and third category had intermediate values and therefore cultivars in this group are considered to be moderately tolerant and moderately susceptible cultivars. In the fourth category, the cultivars had higher values of susceptibility indices and are susceptible to ozone stress (Susceptible group). Therefore, out of 40 Amaranthus cultivars studied, 12 were ozone tolerant, 14 were moderately tolerant and 14 were ozone susceptible cultivars.

## Conclusion

The results of the present study showed that Amaranthus cultivars exhibited variable response to ozone stress. Some cultivars exhibited high foliar ozone injury and low grain yield loss and vice versa. The cultivars response with respect to foliar ozone injury does not always lead to grain yield reductions. Therefore for screening of ozone tolerant and susceptible cultivars, foliar injury alone is not a true criteria and screening study should be based on long term ozone exposure as short term exposure studies would not represent true categorization of cultivars. Though many cultivars were able to tolerate ozone stress but only few were able to minimize its negative impact on its grain yield potential. Since the tolerance for ozone is based on the genetic makeup of cultivar, the identification of ozone tolerant cultivars may be useful in future breeding programs for producing high yielding commercial varieties of Amaranthus to mitigate ozone related yield loss and enhance profitability of the farmers. Since the results reported here are from one year study, more detailed multiyear studies at physiological and biochemical level are needed to quantify the effects of ozone pollution on Amaranthus crop.

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### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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