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Short communication

Mixed cropping has the potential to enhance flood tolerance of drought-adapted grain crops

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ANOVA, analysis of variance
RGR, relative growth rate
ROL, radial oxygen loss

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ABSTRACT

Recently, the occurrences of extreme flooding and drought, often in the same areas, have increased due to climate change. Wetland plant species are known to oxygenate their rhizospheres by releasing oxygen (O_2) from their roots. We tested the hypothesis that wetland species could help upland species under flood conditions; that is, O_2 released from the wetland crop roots would ameliorate rhizosphere O_2-deficient stress and hence facilitate upland crop root function. Flooding tolerance of upland-adapted staple crops—pearl millet (*Pennisetum glaucum*) and sorghum (*Sorghum bicolor*) mix-cropped with rice (*Oryza* spp.) was investigated in glasshouse and laboratory. We found a phenomenon that strengthens the flood tolerance of upland crops when two species—one wetland and one drought tolerant—were grown using the mixed cropping technique that results in close tangling of their root systems. This technique improved the photosynthetic and transpiration rates of upland crops subjected to flood stress (O_2-deficient nutrient culture). Shoot relative growth rates during the flooding period (24 days) tended to be higher under mixed cropping compared with single cropping. Radial oxygen loss from the wetland crop roots might be contributed to the phenomenon observed. Mixed cropping of wet and dryland crops is a new concept that has the potential to overcome flood stress under variable environmental conditions.

1. Introduction

Various grain food crops adapted to environments with different water availability are used worldwide to provide food for humans (and animals). For example, pearl millet and sorghum have been used since ancient times because they are dryland-adapted food crops with high yield potential even under limited rainfall conditions (Rachie and Majmudar, 1980). They are the most widely cultivated grain crops in the semi-arid regions of Asia and Africa. More specifically, they are the major food crops in the Sahel of Africa, which account for more than 70% of all cereals grown in that region (Bhattacharjee et al., 2011). Although pearl millet is one of the most drought-resistant food crops, it is extremely susceptible to conditions caused by waterlogged soil (Zegada-Lizarazu and Iijima, 2005). Daily rainfall intensity and dry-spell duration have significantly increased in South and West African countries during 1961–2000 (New et al., 2006). The latest Intergovernmental Panel on Climate Change (IPCC) report also indicated that extreme precipitation events such as droughts and heavy rainfall have become more frequent throughout eastern Africa during the last 30–60 years (Lyon and DeWitt, 2012; Niang et al., 2014). Moreover, in case of southwest African country of Namibia, they have received to three episodes of substantial flooding (Mendelsohn et al., 2013) and two episodes of severe drought in the north central densely populated region since 2008. This has caused substantial loss of pearl millet production. Food shortages caused by flooding have become common in the semi-arid African regions in the recent years (Bhattacharjee et al., 2011; Newsham and Thomas, 2011).

Rice, the only flood-adapted major cereal, sequentially forms air channels in its root cortex (called aerenchyma) during root growth, and oxygen (O_2) is continuously supplied via the aerenchyma from the shoot to the root apical meristem when the soil is submerged in water. A barrier to radial oxygen loss (ROL) at the root surface helps in providing O_2 to the root apex, but this barrier will not be developed in the newly formed, young meristem regions.

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Therefore, O_2 is released into the rhizosphere near the root tips (Armstrong, 1979; Colmer, 2003; Yamauchi et al., 2013). Upland cereal species adapted to dry environments also form continuous channels for O_2 transport in their cortex tissues, but the amount of gas space is mostly much less than in wetland species (Armstrong, 1979; Justin and Armstrong, 1987), and as a result, their roots may not survive for long in flooded soil. If the O_2 released from the rice root tips can ameliorate rhizosphere O_2 deficiency stress for companion drought-adapted upland grain crops, flood-stressed grain production may become possible in drought-prone areas.

Major grain crops are occasionally mix-cropped with other species for risk management (Wolfe, 2000; Brooker et al., 2014). Mixed cropping of cereals and legumes is a commonly practiced cultivation technique worldwide because of the complementary effects for cereals, which benefit from utilizing the nitrogen that is biologically fixed by the legumes (Ehrmann and Ritz, 2014). Mixed cropping of wet- and dryland-adapted species may overcome the limitations imposed by both flood and drought conditions; Although similar combination has already been discussed before {For example, Setter and Belford (1990)}, there is no experimental evidence for this concept so far. In this study, we tested the hypothesis that wetland species could help upland species under O_2-deficient conditions.

2. Materials and methods

2.1. Plant materials

Pearl millet (*Pennisetum glaucum* cv. Okashana 2) and sorghum (*Sorghum bicolor* cv. Macia), which are adapted for drought-prone semi-arid environments, were used as the upland crops (hereinafter called ‘millets’). These are recommended crop cultivars widely grown in semi-arid southern African countries, as drought-tolerant crops. Rice (*Oryza sativa* cv. Nipponbare and interspecies of *O. sativa* and *Oryza glaberrima* cv. NERICA4) was used as the flood-tolerant crop. Nipponbare is extensively used as an experimental material in Japan. NERICA4 is an upland cultivar being promoted in West African countries such as Benin, Guinea, and Mali for cultivation.

2.2. Pre-germination

Seeds were surface-sterilised with 2.5% (v/v) sodium hypochlorite for 5 min and rinsed in running water for 20 min. Rice seeds were soaked in distilled water, whereas the pearl millet and sorghum seeds were sown on paper towels in Petri dishes. The seeds were pre-germinated in a dark incubator at 30 °C for 14, 24, and 48 h for pearl millet, sorghum, and rice, respectively.

2.3. Seedling establishment

Pre-germinated seeds were sown in a growing medium (Metro-Mix 250 Series; peat moss 43–47%, vermiculite 41–45%, bark ash 3–7%, pearlite 5–9%; N, P_2O_5, K_2O = 60, 70, 100 mg L^{-1}; Sun GRO® Horticulture, USA) in cell trays. One (single-species crop) or two (mixed cropping rice and upland crop) seeds were planted per cell (37 mm long × 37 mm wide × 46 mm deep, with a 5-mm diameter hole at the bottom). The cells were placed on plastic trays and maintained in 3–5-mm deep water. The seedlings were grown in a plant-growth room having 28/23 °C day/night temperature, 14-h photoperiod, and $318 \pm 2\ \mu mol\ m^{-2}\ s^{-1}$ photosynthetically active radiation above the leaf canopy. Seeds of millets were relay-planted into the same cell compartments containing the rice seedlings at 1 week after the rice seeds were sown. A week later (14 days after sowing the rice seeds), the seedlings used in Exp. 1, were transferred to a natural sunlight glasshouse with lighting supplemented by metal halide lamps to extend the photoperiod to 14 h. The temperature was controlled within 25–30 °C by using a gas heating system and automatic roof and window opening system. The cell trays containing the seedlings were cut into individual cell compartments, and then placed on plastic disposal dishes (80 × 80 × 25 mm, length × width × height) to allow the mixed-seedlings to form entangled root mats as shown in Fig. 1. The mixed-seedlings were grown for 3 weeks on hydroponic boxes containing 30 L (maximum depth, 15 mm) of Hoagland solution (Hoagland and Arnon, 1950) circulated at about 2.4 L min^{-1} for continuous O_2 supply. The nutrient solution was at quarter, half, and full strength concentrations during the first, second, and third weeks, respectively. Light penetration to the root mats was prevented by covering the cell trays with grey plastic mulch leaving the plant shoots exposed. The culture was renewed every 3–4 days, and the pH was adjusted and maintained at 6.5 during nursery and subsequent experimental periods. The photosynthetically active radiation in early morning and late afternoon, and midday was 142–464 and 386–947 $\mu mol\ m^{-2}\ s^{-1}$, respectively.

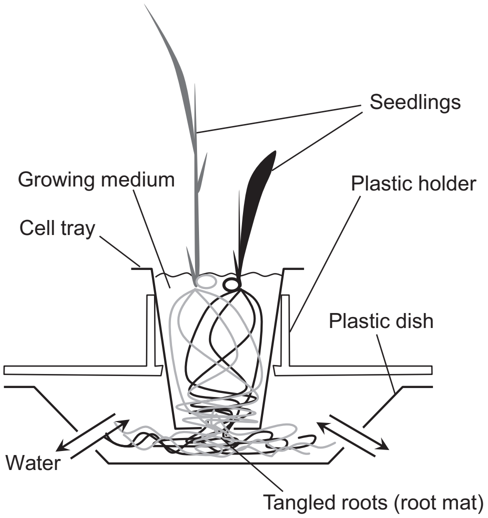

Fig. 1. Schematic diagram of the mixed-seedling system to enhance the intertwining of the roots of the two species (Exp.1). Pre-germinated seeds of pearl millet or sorghum were relay-planted into the same cell compartments containing the rice seedlings at 1 week after the rice seeds were sown. Fourteen days after sowing the rice seeds, all cell trays containing the seedlings were cut into individual cell compartments, which were placed on plastic dishes, the seedlings were then grown further under hydroponic nutrient culture to form entangled root mats.

2.4. Physiological response of the mixed-seedlings under O_2-deficient nutrient culture (Exp. 1)

The effects of an O_2-deficient root environment on the physiological status of the mixed-seedlings were investigated in glasshouse experiments. This experiment was conducted at Kinki University, Japan (34° 40′ N, 135° 44′ E, 172 m ALT), which is located in a temperate climate zone. Four-week-old millet and 5-week-old rice seedlings were transplanted into plastic boxes filled with either air-bubbled (control) or N_2-bubbled (flooded/hypoxic) Hoagland solution (40 L) at full strength and grown for 24 days. The shoots of the mixed-seedlings were fixed into holes drilled through floating Styrofoam platforms fitted in each box. Diffusion of atmospheric gases into the culture medium was minimised by sealing the spaces between the platform and the box and around the planting holes. Hypoxic condition ($6 \pm 0.2\ \mu M\ O_2$), defined as “flood stress”, was induced by daily bubbling of the culture solution with N_2 gas flowing at 6 L min^{-1} for 20 min, whereas air-bubbling was done