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Soil ammonia volatilization following urea application suppresses root hair formation and reduces seed germination in six wheat varieties

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ABSTRACT

Poor seed germination and early seedling growth caused by ammonia toxicity following urea application are major constraints for wheat production. This study aims to determine ammonia volatilization caused by urea and its damage to seed germination, early seedling growth, and its associated physiological mechanism. Two petri dish experiments were conducted under four nitrogen (N) application rates (0, 0.58, 1.16 and 1.75 mg N g^{-1} soil) for six wheat varieties that differ in their ammonia tolerance with regard to the germination percentage (%), its associated indicator, related growth parameters, root hair development and changes in the content of endogenous hormones. Increasing the N rate significantly increased ammonia volatilization from 9.69 to 121.9 mg per pot and therefore reduced the germination percentage (%) by 51%–95% and suppressed root and shoot growth ($P \leq 0.05$). The variation in the response of the different genotypes to ammonia toxicity was distinguishing. Root was more sensitive to ammonia volatilization than shoot. Ammonia toxicity seriously suppressed root hair formation, and the varieties that were tolerant of ammonia toxicity could sustain relatively more abundant root hair formation compared with the other more sensitive varieties. The specific balance between several endogenous plant hormones was also found in response to ammonia toxicity between two varieties with contrasting tolerance. This study emphasized the potential to select varieties with high tolerance to ammonia toxicity to improve seed germination and early seedling establishment through a breeding programme. The physiological mechanisms of seed germination responsible for high tolerance to ammonia toxicity are rapid root hair formation and the synergistic effect between several endogenous plant hormones.

1. Introduction

Nitrogen (N) fertilizer use has increased almost fivefold since 1960, and it has made a great contribution to global food security (Wu and Ma, 2015). Urea is one of the most popular synthetic N fertilizers, accounting for over 50% of all N applications (Glibert et al., 2006), because of its high solubility, high N content, low cost, and ease of handling. Although it has been widely used in agriculture worldwide, there are still major problems relevant to its use, which have been addressed several decades ago (Bremner, 1995; Bremner and Krogmeier, 1988, 1989).

When urea is applied to the soil, it is rapidly hydrolysed by urease enzymes to ammonia and carbon dioxide, with a corresponding increase in soil pH (Bremner and Krogmeier, 1989). The excessive accumulation of ammonia can lead to serious toxicity for seed germination and poor seedling growth (Buresh, 1987; Bremner and Krogmeier, 1988; Haden et al., 2011b). Toxicity arises when gaseous ammonia produced by urea hydrolysis spreads passively into plant cells, where it disturbs cellular metabolism by interfering with the regulation of intracellular pH between the cytosol and vacuoles (Kosegarten et al., 1997). Symptoms related to ammonia toxicity include a lower germination percentage (%), damage to root and the inhibition of seedling growth (Bremner, 1995). Toxicity becomes even worse when ammonium fertilizers are broadcast or banded in close proximity to sown seeds (Buresh, 1987; Fan and Mackenzie, 1995; Rochette et al., 2009).

Wheat (*Triticum aestivum* L.) is the second most widely produced crop in the world, and its production is considered vital for global food security (FAO, 2006). Good seed germination and

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the subsequent uniform establishment of healthy seedlings are critical factors for wheat production and are of both economic and ecologic importance (Miransari and Smith, 2014). Because seed germination is sensitive to any abiotic or biotic stress, the germination process is considered the most critical phase in the crop life cycle.

In this regard, agronomists always attempt to obtain rapid and uniform germination and seedling emergence once seeds are sown in the field because the delayed germination of the seeds is not recovered and will reduce the plant population and the crop yield. There are many possible reasons for poor seed germination, such as improper seed sowing depth, drought stress, tillage method, and soil chemical properties (Li et al., 2008; Haden et al., 2011b; Qi et al., 2011). Among those reasons, ammonia toxicity due to urea application is one of the most important factors (Bremner, 1995) and has become more critical in recent years.

N fertilizer, in terms of the urea applied along with seeds on the sowing date, is universal and widespread in most wheat–producing areas in China due to its simplicity, the reduction in labour and convenience to mechanization (Wu and Uphoff, 2015). Total N in the form of urea was generally applied on the sowing date, rather than in the proper manner with several split–applied patterns, which has resulted in poor synchronization between the soil N supply and the crop demand, resulting in a large amount of inorganic N being available in the soil, which causes high ammonia volatilization during seed germination process (Wu and Ma, 2015). This cultivation practice magnifies the toxicity of ammonia volatilization on seed germination after urea application.

Plant roots provide plants with water and nutrients as well as anchorage and play an important role in plant life (Kong et al., 2014). Under abiotic stress, the root is the initial perceiver of stress, followed by a series of adaptive responses at the physiological, cellular and morphological levels (Ghosh and Xu, 2014; Kwasniewski et al., 2016). Root hairs in plants contribute significantly to the surface area and exploitable volume by increasing the efficient absorption of water and mineral nutrients (Peterson and Farquhar, 1996; Raven and Edwards, 2001). Maintaining root system elongation and root hair development plays an essential role in alleviating abiotic stress (Kwasniewski et al., 2016). However, there are few reports that investigate the adverse effect of ammonia toxicity on root elongation and root hair formation. Recently, the quantification of morphological traits of root hairs has become easier through the use of high–resource image analysis; therefore, the role of root hair formation could be determined more effectively.

Plant hormones, which are involved in many physiological and biochemical processes of plants, significantly regulated most plant activities, including seed dormancy and germination, as well as the establishment of the seedling (Shu et al., 2016; Rajjou et al., 2012). The endogenous abscisic acid (ABA) content is down–regulated and the gibberellic acid (GA) content is up–regulated with seed germination for the imbibition and stratification treatments (Vanstraelen and Eva, 2012). GA can enhance amylase activity and promote seed germination, beside this, the indole–3–acetic acid (IAA) and zeatin (Z) and zeatin riboside (ZR) also promote seedling growth by regulated cell division and elongation (Miransari and Smith, 2014). The response of plant hormones to abiotic stress often involves not just one type of hormone but a variety of hormones that creates a synergistic effect, that is, an integrated balanced effect of hormones (Miransari and Smith, 2014; Vanstraelen and Eva, 2012; Lopez-Bucio et al., 2002). However, there are limited studies regarding the changes in endogenous hormones under ammonia toxicity conditions during the wheat seed germination process.

This study aimed to (1) determine the ammonia volatilization of urea and its damage to seed germination and early seedling growth in six wheat varieties when urea was applied at different rates; (2) verify the adverse effect of ammonia toxicity on root hair formation and its genotypic variation; and (3) explore the integrated balance of the endogenous hormone content in response to ammonia toxicity.

2. Material and methods

2.1. Soil material

Soil samples were collected from the top soil (0–25 cm) of field, where winter wheat was grown in the Crop Specimen Farm of Northwest A&F University, Shanxi Province. The soil type belongs to clay loam. Physical and chemical properties of the soil samples were listed in Table 1. Before using, soil sample was air–dried, pulverized, crushed to pass through a 1.5–mm screen and mixed to homogenize the large sample.

2.2. Treatments and experimental design

Petri dish experiment I was conducted to determine the ammonia volatilization in response to different N rates, using the method of Bremner and Krogmeier (1989) with some modification. An 80 g sample (dry weight basis) of soil was placed in a large plastic petri dish (150 × 15 mm). The soil was then moistened with either 10 ml distilled water (zero N control) or 10 ml water containing dissolved urea at three N rates (0.58, 1.16 and 1.75 mg N g^{-1} soil). A smaller uncovered petri dish (60 × 10 mm) containing 10 ml 4% boric acid and 1–2 drops of mixture indicators (bromocresol green and methyl red) were placed inside the larger petri dish to act as a trap for absorbing ammonia gas. The lid of the larger dish was sealed tightly, and therefore gaseous ammonia could diffuse through the air and interact with the surface of the acid trap without being lost from the incubation container.

Each dish was incubated in darkened incubator for 4 days at 25 °C with humidity of 75%. Within the incubator completely randomized design was used with four replicates for four N treatments. The amount of ammonia volatilized from soil was determined by titrating the boric acid using 0.01N HCl (Bremner and Krogmeier, 1988, 1989).

In Petri dish experiment II, the N application rates and the incubation method were the same to the incubation experiment I. Twenty presoaked (for 6 h) wheat seeds were placed in a small petri dish containing moistened filter paper, instead of 4% boric acid. This small dish was placed inside the larger dish, which was then covered with a lid to prevent ammonia gas losses, as described above. In this incubation, the seeds were not in contact with the soil but remained exposed to any ammonia gas being released from it. Treatments were arranged in a split plot design with N application rates as the main–plot and variety as sub–plot. There were four replications for each treatment. The main treatment consisted of four N rates, as described in experiment I. The sub–treatment included six varieties (Xinong979, Xiza5, Jinmai47, Wumai148, Changhan58 and Changhan343). The petri dishes were incubated under identical conditions described earlier, until the 4th day when finally constant seedling emergence percentage was measured and all seedlings were sampled for measuring the seed germination and several morphological

Table 1
Soil chemical properties of the top soil.

Organic C (g/kg)	pH	Olsen P (mg/kg)	Available K (mg/kg)	Total N (g/kg)	Total P (g/kg)	Total K (g/kg)
12.3	8.2	6.1	188	0.79	0.71	14.6