



Full Length Article

Influence of Nitrogen and Phosphorus Fertilization on Quality and Germination Potential of Smooth Bromegrass Seed

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Abstract

A field experiment was conducted to evaluate the effects of two nitrogen levels (N: 0, 100 kg ha⁻¹) and four phosphorus levels (P: 0, 60, 90, 120 kg P₂O₅ ha⁻¹) on smooth bromegrass (*Bromus inermis* Leyss.) seed quality by measuring germination, mean germination time (MGT), protein and phosphorus contents, acid phosphoesterase (APT) and antioxidant enzymes activities, hydrogen peroxide (H₂O₂) and malondialdehyde (MDA) contents and electrical conductivity. Nitrogen application improved seed protein content and reduced P content at different P application levels, while there was a contradicted effect for P application on increasing seed P content and decreasing protein content. Mean germination time of seeds harvested from 100 kg N ha⁻¹ treatment was significantly ($P < 0.05$) prolonged, indicating that N application could delay seed germination rate. Phosphorus deficiency or excessive P application level would also delay the initiation of germination. Seed-aging assays showed that N application could decrease relative loss of germination (RLG) through improving CAT and APT activities and reducing the content of H₂O₂ and MDA at the lower P application. From the findings of the present study, optimum application ratio of N and P improved germinability and longevity of smooth bromegrass seed. © 2018 Friends Science Publishers

Keywords: Seed vigor; Fertilizer; Germination; Smooth bromegrass

Introduction

Seed quality is vital to agricultural systems, with early seedling growth and establishment especially dependent on seed reserves, before photosynthetic capacity is developed. Seed size, weight, protein, carbohydrates, oil and mineral contents are closely linked with seed quality (Marco and De, 1990; Smart and Moser, 1999; Snider *et al.*, 2016; Sousa *et al.*, 2016). Although seed quality is affected by many factors, fertilizer management is especially important. Deficiency of any one of the macronutrients will significantly hamper plant growth and reduce seed yield and quality (Austin, 1966). Although some studies have been conducted to explore the influence of N and P on seed quality, including those of grains (Warraich *et al.*, 2002; Arduini *et al.*, 2006), legumes (Uddin *et al.*, 2014) and oil seeds (Oskouie and Divsalar, 2011; Nosheen *et al.*, 2016), few have examined the influence on grass seed quality.

Nitrogen is the largest applied nutrient in all areas of the world and the most limiting cause of production for grass-seed crops compared to legume-seed crops (Fairey and Lefkovitch, 1998; Malhi *et al.*, 2001). Researchers have proved that N fertilizer is related to susceptibility of mother plant, seed yield and seed quality (Ros *et al.*, 2008; Malhi *et al.*, 2014; Huang *et al.*, 2016). Under N deficiency, plant tends to allocate more photosynthates to roots than shoots,

hindering vegetative growth and seed yield (Bacon, 1995). Nevertheless, excess of N application can lead to unfavorable-delayed senescence and maturity (Sawan *et al.*, 2009). Application of N can lead to differences in crop seed germination and nutrient content (Fallahi *et al.*, 2013). Nitrogen application significantly increased wheat (*Triticum aestivum* L.) seed final germination percentage and protein content (Warraich *et al.*, 2002; Svecnjak *et al.*, 2007), while mean germination time (MGT) and phosphorus percentage were significantly reduced with N application (Warraich *et al.*, 2002). Seed protein content was related to germination time and higher protein content could delay the start of germination (Pettersson, 2007). In soybean (*Glycine max* L.) seeds, germination percentage decreased as protein level increased due to imbibitional injury, which is the physical injury caused by rapid water imbibition of low moisture seeds (Levan *et al.*, 2008). However, little work has been done to the effect of N fertilization on grass seed quality.

Phosphorus (P) is essential component of adenosine triphosphate and forms the skeleton of cellular membrane and DNA. Its application to mother plant significantly influenced total P content in seeds (Marco and De, 1990; Modi, 2002). Total P content in seeds was highly correlated with phytic acid, the main form of storage phosphorus and related to metabolic functions in seeds (Batten, 1986; Raboy, 2009). Seed phosphorus reserves were rapidly

mobilized during germination and translocated to emerging root and shoot tissues (White and Veneklaas, 2012). Seeds with high P content showed faster germination, larger first leave and longer roots (Marco and De, 1990; Zhu and Smith, 2001). Furthermore, P application seemed to change seed protein and lipid content (Munamava *et al.*, 2004; Galavi *et al.*, 2011; Krueger *et al.*, 2012).

Smooth brome grass (*Bromus inermis* Leyss.) is a kind of perennial and rhizomatous grass with high forage value and productivity (Liu *et al.*, 2014). It is characterized by a high tolerance to drought and low temperatures and a medium resistance to soil salinity, this species has already been introduced for restoring degraded grassland and establishment of artificial pasture (Liu *et al.*, 2008; Antonova *et al.*, 2015). Breeding of smooth brome grass has focused on increasing biomass and seed yield (Wang *et al.*, 2012). Little is known about whether seed quality was affected by N and P fertilization. Therefore, this study was conducted to explore the impact of N and P application on seed germination, protein content and aged physiological reactions to improve germinability and longevity through fertilization management.

Material and Methods

Field Conditions and Treatments

Field experiments were conducted during 2013 and 2014 at the Grassland Research Station of China Agricultural University, located in Yuershan farm, Hebei province, China (41°44' N, 140°16' E and 1455 m altitude). The experimental site soil type is a Lithic Haprendoll (a stony mollisol with a mollic epipedon less than 50 cm thick, a udic moisture regime, and soil temperature regime warmer than cryic) (USDA-NRCS, 2010). Initial soil chemical characteristics (0–30 cm) were: pH 7.70; 30.55 g kg⁻¹ organic matter, 96.42 mg kg⁻¹ available N; 3.80 mg kg⁻¹ available P; 22.80 mg kg⁻¹ available K. The available N was determined by 1.07 M sodium hydroxide (NaOH) and iron sulfate (FeSO₄) powder at 40°C for 24 h, and then absorbed with 2% (w/v) boric acid (H₃BO₃) and titrated with 0.005 M sulfuric acid (H₂SO₄) (Bao, 2000; Xiong *et al.*, 2008). The available P was determined by sodium bicarbonate (NaHCO₃) extraction and subsequent colorimetric analysis (Olsen and Sommers, 1982). Available K was determined using an ammonium acetate extraction followed by emission spectrometry (Knudsen *et al.*, 1982).

Field plots were established on 22 July, 2013, planting smooth brome grass seeds at 35 kg ha⁻¹ (seed purity 98% and germination percentage 87%) with a 75 cm row spacing. The experiment was a completely randomized split-plot design with four replications in which N applications were placed in main plots and P applications were kept in sub-plots. Fertilizer treatments were established with two N rates: N0 (0 kg N ha⁻¹) and N100 (100 kg N ha⁻¹) and four phosphorus rates: P0 (0 kg P₂O₅ ha⁻¹), P60 (60 kg P₂O₅ ha⁻¹), P90 (90 kg P₂O₅ ha⁻¹) and P120 (120 kg P₂O₅ ha⁻¹). Nitrogen

was applied as urea on 16 May, 2014 and P as P₂O₅ on 22 July, 2013. No irrigation was supplied and weeds were removed by hand. There were no other management factors imposed. Seeds were harvested by hand on July 28, 2014.

Determination of Seed Moisture Content

Seed moisture content was measured according to ISTA protocol (2015). Approximately 4.5 g of seed was placed in a pre-dried sample container, dried at 130°C for one hour, then cooled for 30–40 min in a desiccator at 25°C and reweighed.

Seed Moisture Content Adjustment

Seeds were equilibrated in a sealed container with saturated KNO₃ solution at 25°C, with 94% relative humidity. Seeds were removed after three days of equilibration and reweighed until the moisture content (MC) reached 16% (w/w). Seeds were then sealed in foil bags, each with 10 g of seed.

Seed Controlled Deterioration

After the adjustment of seed moisture content and sealing in foil bags, seed sample bags were immersed in a thermostatically controlled water bath at 45°C for six days, after which the seed samples were stored at 4°C awaiting analysis.

Seed Germination Assay

A seed germination assay was performed according to ISTA protocol (2015). Petri dishes (12 cm diameter) were lined with three layers of filter paper (Guangda Company, China), moistened with 12 mL distilled water, and incubated in a growth chamber set to 15/25°C under a 16 h dark/8 h light photoperiod. Each treatment was replicated four times, with 50 seeds in each petri dish. Germinated seed counts (defined as when the length of the radicle was > 2 mm) were made every 6 h between 48 h and 96 h after imbibition for calculation of mean germination time (MGT). Germination percentage (G) was determined on the 14th day following incubation. The relative loss of germination percentage (RLG) was calculated as the ratio of the loss of germination percentage caused by deterioration (Gd) and the germination percentage of unaged seeds (G):

$$G(Gd) = (G14/N) \times 100\%$$

$$MGT = \frac{\sum(nt)}{\sum n}$$

$$RLG = \frac{G - Gd}{G} \times 100\%$$

G14 = number of normal seedlings at the end of the 14th day

N = number of tested seeds

t = number of hours after imbibition

n = number of germinated seeds during t hours

∑n = total number of germinated seed.

Determination of Crude protein and P Content

Total N content of smooth bromegrass seed was determined using the Kjeldahl N method and crude protein content was estimated by multiplying N content by 6.25 (since plant protein contains ~16% N) (Association of Official Analytical Chemists, 1990). P content was determined by the Mo-Sb colorimetric method (Lu, 2009).

Determination of Seed Electrical Conductivity

To determine electrical conductivity, four replications of unbroken, uniform, aged seeds (0.3 g, MC: 16%) were selected for electrical conductivity assay. Seeds were rinsed three times with deionized water and incubated in 150 mL deionized water at 20°C for 24 h in darkness. Seed exudate was measured for electrical conductivity using a DDSJ-308A conductivity meter (Shanghai, China) (Muasya *et al.*, 2006).

Enzyme Extraction and Assays

To extract antioxidant enzymes, four replicates of aged seeds (0.3 g, MC: 16%) were ground in 6 mL, 50 mM phosphate buffer (including 1.0 mM EDTA, 1% PVP, pH 7.0) using a mortar and pestle. Homogenates were transferred into 10 mL plastic centrifuge tubes and centrifuged at 12000 rpm for 20 min. All extract processes were conducted at 4°C. Resulting supernatants were used to assay antioxidant enzymes activities.

Catalase (CAT) activity was measured according to its ability to decompose H₂O₂ and resulting decline of H₂O₂ absorbance at 240 nm. Supernatant (50 µL) was mixed with 3.4 mL of 25 mM phosphate buffer (including 0.1 mM EDTA, pH 7.0), and 200 µL of 100 mM H₂O₂. Absorbance changes were measured for 2 min immediately after the addition of H₂O₂.

Superoxide dismutase (SOD) activity was measured according to the method of Rao and Sresty (2000) in which 1.5 mL of 50 mM phosphate buffer (pH 7.8), 0.3 mL of 130 mM methionine, 0.3 mL of 750 µM nitroblue tetrazolium (NBT), 0.3 mL of 100 µM EDTA, and 0.3 mL of 20 µM riboflavin were mixed with 0.1 mL supernatant. Absorbance was measured at 560 nm. One unit of SOD activity was defined as the suppression of 50% NBT photochemical reduction.

Determination of Acid Phosphoesterase (APT) Activity

Four replicates of aged seeds (0.3 g, MC: 16%) were ground in 5 mL of 50 mM tris buffer (pH 7.5). Homogenates were centrifuged at 4000 g for 10 min. Then, 0.1 mL of supernatant was diluted to 2 mL followed by the addition of 0.2 mL sodium MBis (4-nitrophenyl) phosphate and incubated at 30°C for 10 min. To stop the reaction, 2 mL of 0.5 M NaOH was added to each tube. Absorbance was measured at 400 nm.

Determination of H₂O₂ and MDA

H₂O₂ content was measured according to the method of Patterson *et al.* (1984). Four replicates of aged seeds (0.2 g, MC: 16%) were ground in 2 mL of 5% trichloroacetic acid solution. Reaction mixtures contained 1 mL supernatant, 0.2 mL ammonium hydroxide, and 0.1 mL of 20% titanium tetrachloride in HCl. Homogenates were then centrifuged at 12000 rpm for 20 min at 4°C. Precipitate was suspended in 3 mL of 1 M H₂SO₄ and absorbance was measured at 415 nm.

MDA content was measured according to the thiobarbituric acid (TBA) colorimetric method (Bailly *et al.*, 1996). Four replicates of aged seeds (0.2 g, MC: 16%) were ground in 8 mL of 5% trichloroacetic acid solution and then centrifuged at 12000 rpm for 20 min. Then, 3 mL supernatant was mixed with 2.5 mL of 5% trichloroacetic acid (containing 0.5% TBA). Samples in centrifuge tubes were suspended in boiling water for 15 min followed by quickly cooling in an ice bath. Following 20 min of 12000 rpm centrifugation, the supernatant absorbance was measured at 600 and 532 nm.

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using SPSS version 19.0. Duncan's new multiple range test ($P < 0.05$) was applied to compare significant treatment means.

Results

Changes of Protein and Phosphorus Content in Collected Seeds with N and P Application Treatments

The results showed that the protein content of smooth bromegrass seeds was significantly ($P < 0.05$) increased by N application at different level of P application (Fig. 1a). However, seed protein content in N0 and N100 conditions both presented the declining trend with P application rate increased from 0 to 120 kg P₂O₅ ha⁻¹. The changes of P content affected by N and P application were different from those of protein content (Fig. 1b). Seed P content increased significantly ($P < 0.05$) with P application rate increasing from 0 to 90 kg P₂O₅ ha⁻¹ in both N0 and N100 level. For collected seeds with same P level, the P content was reduced significantly ($P < 0.05$) by N100 treatment.

Changes of MGT and Germinability in Collected Seeds with N and P Application Treatments

Mean germination time of seeds from N100 treatment was significantly ($P < 0.05$) higher than those from N0 treatment, which indicated that N application could prolong the lag period of seed germination under different P level (Fig. 2a).

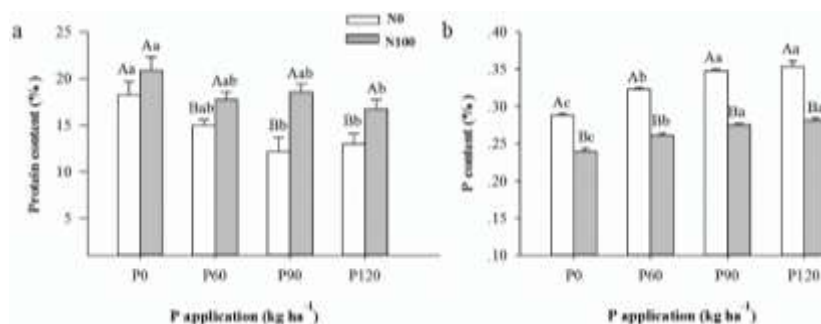


Fig. 1: Effects of N and P application on protein (a) and P (b) content in harvested seeds of smooth brome grass. Means with different letters indicate statistical difference at 0.05 level. Different lowercase letters refer to significant difference at P level, while different capital letters indicate significant difference at N level

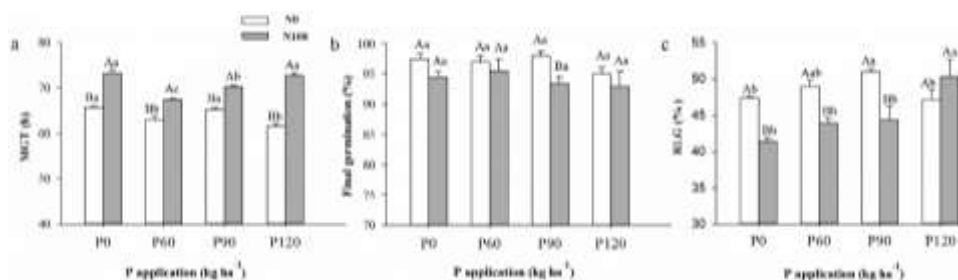


Fig. 2: Effects of N and P application on mean germination time (a), final germination (b) and relative loss of germination (c) in harvested seeds of smooth brome grass. Means with different letters indicate statistical difference at 0.05 level. Different lowercase letters refer to significant difference at P level, while different capital letters indicate significant difference at N level

Under N0 condition, MGT of seeds from P60 and P120 treatments significantly decreased than those from P0 and P90. However, for N100, MGT of seeds from P60 and P90 decreased than those from P0 and P120. Final germination of seeds from N application both presented the higher level (over 93%), and there was no significantly ($P>0.05$) difference among different P treatments (Fig. 2b). However, final germination of seeds from N100 was lower than that from N0 under P90 condition. Relative loss of germination (RLG) of seeds from N100 treatment significantly ($P<0.05$) decreased with compare to those from N0 at 0, 60 and 90 kg P_2O_5 ha⁻¹, but there was no significant ($P>0.05$) difference between them at 120 kg P_2O_5 ha⁻¹ (Fig. 2c). Changes of RLG of seeds from N100 treatment presented a different trend with those from N0 under different P applications. RLG from N100 increased significantly ($P<0.05$) from P90 to P120 but from N0 decreased significantly ($P<0.05$).

Changes of Enzyme Activities in Aged Seeds with N and P Application Treatments

There were no significant differences for CAT or SOD activities among seeds with different P application (Fig. 3). Compared with N0 treatment, CAT activities of seeds from N100 significantly ($P<0.05$) increased at application of 60 and 90 kg P_2O_5 ha⁻¹ (Fig. 3a), but SOD activity of seeds

from N100 significantly ($P<0.05$) improved at application of 120 kg P_2O_5 ha⁻¹ (Fig. 3b). On the other hand, there was no significant ($P>0.05$) difference for acid phosphoesterase activities of seeds from N0 treatment among the level of P application, but activity of this enzyme attained the maximum at 60 kg P_2O_5 ha⁻¹ application and exhibited the significantly ($P<0.05$) declining from 90 to 120 kg P_2O_5 ha⁻¹ application. Compared with N0 treatment, acid phosphoesterase activity of seeds from N100 significantly ($P<0.05$) increased at 60 kg P_2O_5 ha⁻¹ application.

Changes of H₂O₂ and MDA Contents of Aged Seeds with N and P Application Treatments

H₂O₂ content of aged seeds was affected by the N and P application presented the different changing tendency (Fig. 4a). For N0 application, H₂O₂ contents of seeds from P0 and P90 treatments were significantly ($P<0.05$) higher than those from P60 and P120. H₂O₂ contents of seeds showed gradually declining from P0 to the minimum at P90 and attained the maximum at P120 under N100 application. Also, H₂O₂ contents of seeds from N100 treatment significantly ($P<0.05$) decreased as P application at the level of 0 and 90 kg P_2O_5 ha⁻¹ with compare to N0 treatment, while it significantly ($P<0.05$) increased at 120 kg P_2O_5 ha⁻¹.

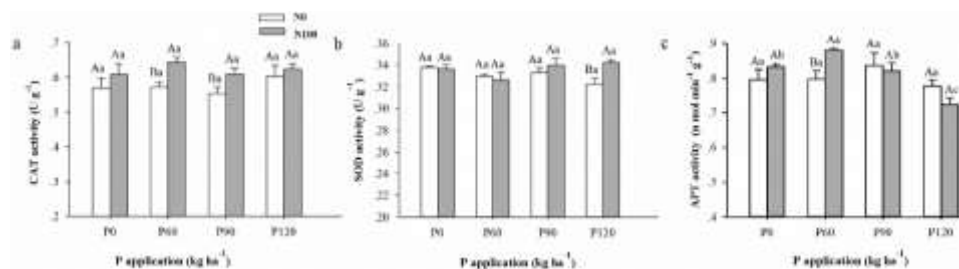


Fig. 3: Effects of N and P application on CAT (a), SOD (b) and acid phosphoesterase enzyme (c) activities in aged seeds of smooth brome grass. Means with different letters indicate statistical difference at 0.05 level. Different lowercase letters refer to significant difference at P level, while different capital letters indicate significant difference at N level

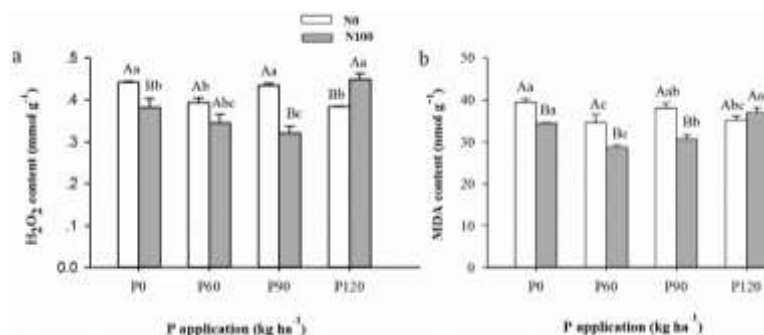


Fig. 4: Effects of N and P application on H₂O₂ (a) and MDA (b) contents in aged seeds of smooth brome grass. Means with different letters indicate statistical difference at 0.05 level. Different lowercase letters refer to significant difference at P level, while different capital letters indicate significant difference at N level

MDA contents of aged seeds from N0 and N100 treatments showed similar changing trend as P application from 0 to 120 kg P₂O₅ ha⁻¹, both reached minimum at the level of 60 kg P₂O₅ ha⁻¹, then increased at 90 kg P₂O₅ ha⁻¹ (Fig. 4b). Also, MDA contents of seeds from N100 decreased as P application at the level of 0, 60 and 90 kg P₂O₅ ha⁻¹ with compare to N0 treatment, while there was no significant difference between them at 120 kg P₂O₅ ha⁻¹.

Changes of Electrical Conductivity of Aged Seeds with N and P Application Treatments

Electrical conductivity of aged seeds was significantly affected by P application. They reached the minimum at the level of 60 kg P₂O₅ ha⁻¹ and then significantly increased at 90 kg P₂O₅ ha⁻¹ for both N0 and N100 treatments (Fig. 5). Also, N100 treatment significantly increased the electrical conductivity of seeds only at the level of 60 kg P₂O₅ ha⁻¹ with compare to N0 treatment. There was no significant difference of electrical conductivity between N0 and N100 treatments at 0, 90 and 120 kg P₂O₅ ha⁻¹.

Discussion

Nitrogen and phosphorus are the essential elements for plant growth and reproduction as the most applied fertilizer around the world. N and P application had positive effects

on total protein and P contents of smooth brome grass seeds respectively, but each of the two elements had antagonistic effect on the other absorption, which are in agreement with previous findings (Ning *et al.*, 2009; Bi *et al.*, 2013; Fallahi *et al.*, 2013). Also it further reminded us to balance N and P fertilization to increase these nutrients uptake efficiency. Proteins are the main forms of nitrogen in seeds which are indispensable reserves and involved in almost all kinds of metabolic activities. Nevertheless, the protein content increased with N application rate among different kind of species (Svecnjak *et al.*, 2007; Malhi *et al.*, 2014), which would cause imbibed injury and delayed seed germination (Pettersson, 2007; Levan *et al.*, 2008). On the other hand, P content of smooth brome grass seed significantly ($P < 0.05$) decreased with N application, while seed P content was reported to positively affect seedling growth and dry matter accumulation (Zhu and Smith, 2001; White and Veneklaas, 2012). At the early stage of seed germination, higher seed P content was able to promote faster initial root growth and seedling establishment (Nadeem *et al.*, 2011; Nadeem *et al.*, 2012; White and Veneklaas, 2012). In this study, MGT maintained the lowest at N0P60 or N100P60 treatments, indicating lower P content would be beneficial for radicle growing fast. MGT could be used to evaluate the level of seed vigor and lower MGT showed seeds were more vigorous and could germinate in less time (Matthews and Hosseini, 2006).

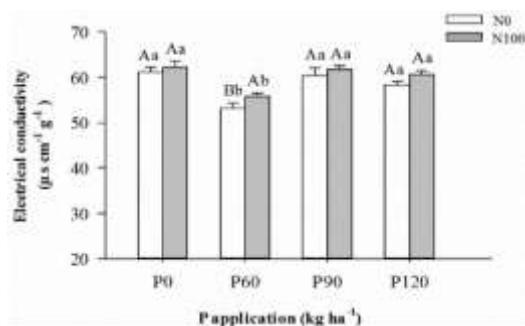


Fig. 5: Effects of N and P application on electrical conductivity in aged seeds of smooth brome grass. Means with different letters indicate statistical difference at 0.05 level. Different lowercase letters refer to significant difference at P level, while different capital letters indicate significant difference at N level

N application (100 kg N ha⁻¹) significantly delayed radicle emergence as indicated by higher MGT in this study, which was opposite to results obtained from wheat, cotton (*Gossypium barbadense* L.) and rape (*Brassica campestris* L.) seed (Warraich *et al.*, 2002; Sawan *et al.*, 2009). The highest MGT of rape seed observed in no-fertilizer application treatment and the lowest was in treatment of 100 kg N ha⁻¹ (Oskouie and Divsalar, 2011), while MGT of wheat seeds was significantly reduced with the N application (Warraich *et al.*, 2002). However, higher P content of smooth brome grass seed adversely delayed radicle emergence, indicating that excessive P level might be detrimental to seed vigor and the application of 60 kg P₂O₅ ha⁻¹ always promoted seedling growth whether N fertilizer was applied or not.

Controlled deterioration (CD) treatment is presumed to mimic nature ageing, which is widely used to evaluate seed vigor, to study the ageing mechanisms during seed storage and predict seed longevity (Larsen *et al.*, 1998; Rajjou and Debeaujon, 2008). In our study, although there were no significant differences ($P < 0.05$) for germination percentage of smooth brome grass seeds among N or P application, RLG decreased as seed from N100 treatments while increased with seed P content increasing. Thus, under the combination of lower P application and higher N application, the lower RLG indicated that smooth brome grass seeds could maintain higher vigor level and got the stronger tolerance for storage.

Seed physiological processes were influenced by protein and P content during smooth brome grass seed ageing period. However, seed protection systems could play the role for allowing them to survive during storage. Antioxidant enzymes system is one of the most important protection systems to keep seed redox status (Kong *et al.*, 2015; Xia *et al.*, 2015). In this study, CAT and acid phosphoesterase activities were significantly ($P < 0.05$) increased by N100P60 treatment. Acid phosphoesterase is a kind of organic phosphate ester hydrolase, the activity of

acid phosphoesterase is closely related to seed vigor which was proved to reduce during seed deterioration (Ma *et al.*, 2009). On the other hand, H₂O₂ and MDA contents of seeds from P application reached the minimum at the level of 60 kg P₂O₅ ha⁻¹, and decreased further as N application. H₂O₂ was reported to be produced and accumulated in dry seeds and over accumulation of H₂O₂ would result in abnormal seedling or even preventing germination (Bailly *et al.*, 2008). MDA, products of lipid peroxidation, was associated with an increase in cell membrane permeability (Farmer and Mueller, 2013). CAT, acid phosphoesterase reaction to CD indicated that N application could decrease RLG through reducing the content of H₂O₂ and MDA at the lower P application. Bailly *et al.* (1998; 2008) showed that the loss of seed vigor was mainly related to the loss of CAT activity and therefore with a decreased capacity of H₂O₂ detoxification thus leading to lipid peroxidation, and the works of our study showed the similar correlation among CAT activity, H₂O₂ and MDA. Furthermore, the results of electrical conductivity measurement illustrated that membrane integrity could be maintained in the seeds from the application of 60 kg P₂O₅ ha⁻¹. Seed membrane permeability increased as seeds deteriorated under nature or artificial accelerated ageing and thus resulted in more leakage of electrolytes after rehydration (Perez and Argüello, 1995; Mao *et al.*, 2008).

It could be suggested that N application would increase seed protein content and reduce seed P content. On the contrary, P application would increase seed P content and decrease seed protein content. Seed germinability of smooth brome grass could be maintained the higher level at the treatment of 100 kg N ha⁻¹ and 60 kg P₂O₅ ha⁻¹, which were illustrated through the lower level of MGT. Phosphorus deficiency or excessive P application level would prolong seed germination. The treatment of 100 kg N ha⁻¹ and 60 kg P₂O₅ ha⁻¹ also increased seed longevity by increasing the level of CAT and acid phosphoesterase activities and decreasing the content of MDA and H₂O₂ of aged seed. So, the field fertilization management not only related with plant hay and seed yield, but also optimum fertilization rate would determine the level of seed germinability and longevity.

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