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How to improve yield and quality of potatoes: effects of two rates of urea N, urease inhibitor and Cytozyme nutritional program

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Abstract

A field experiment was conducted to assess the efficiency of urea applied with urease inhibitor [N-(n-butyl) thiophosphoric triamide (nBTPT- trade-name Agrotain®), and organic compound (Cytozyme) in minimizing abiotic plant stress in a potato (*Solanum tuberosum* L.) in Dera Ismail Khan, Pakistan in 2010-2011. The nine treatments of control (no N or Cytozyme), urea applied at 200 and 300 kg N ha^{-1}, Agrotain treated urea applied at 200 and 300 kg N ha^{-1}, urea-200+Cytozyme, urea-300+Cytozyme, Agrotain treated urea-200+Cytozyme, and Agrotain treated urea-300+Cytozyme, were replicated 5 times. Potato growth, yield and quality were significantly influenced by urea applied with Agrotain and Cytozyme. Agrotain treated-urea-200 with Cytozyme resulted in maximum plant survival (91%), plant height (48 cm), plant canopy (61 cm) and the number of stems per plant (3.9 stems) compared to urea alone. Agrotain-treated urea applied at 200 and 300 kg N ha^{-1} increased potato yield by 46% and 42%, respectively, compared to urea alone. Cytozyme with urea @ 200 and 300 kg N ha^{-1} increased potato yield by 53% and 35%, respectively, comparing to potato crops receiving urea at the two N rates. Tuber yield improved by 14% when Cytozyme was applied with Agrotain-treated urea at 200 kg N ha^{-1}. Cytozyme and urea applied with Agrotain treated urea-300+Cytozyme produced 33% of large tubers, followed by 31% of medium tubers with urea-200 and Agrotain treated urea-200+Cytozyme. Our results demonstrate that urea applied at 200 kg N ha^{-1} with either Agrotain or with Cytozyme have the most potential to enhance potato yield.

Keywords: Agrotain, Cytozyme, potato yield, quality, urea

1. Introduction

Potato (*Solanum tuberosum* L.) is one of the four most important crops in the world, especially in India, Pakistan, and Afghanistan. According to the FAO 2011 statistics, potato production worldwide was estimated to be 314 million tons in 2009. Potato is a native crop of South America and was first cultivated by the Incas. It was introduced by early Spanish explorers into Europe during the 16th century, but has been cultivated as a food crop since late 17th century.

In Pakistan, potato is regarded an important vegetable crop consumed under many dishes. In Khyber Pakhtunkhawa, potato is mostly grown in northern hilly areas, as well as on the plains, during autumn, spring and summer. This is due to the fact that a wide range of favourable climatic conditions (rainfall, temperature, soil radiation and soil moisture), and different altitudes can be found in these areas. These regions include parts of upper Swat, Kalaam, Bahrain, Mansehra, Balaakot, Kaghan, Naran, Abbotabad, Peshawar and Dera Ismail Khan.

Potato is a short-cycle crop, requiring sufficient macro- and micro-nutrients fertilization and irrigation for optimum plant growth (from vegetative stages to tuber formation and maturity). Among the macro-nutrients, nitrogen (N) generally represents the greatest limitation in potato production (Hendrickson and Douglass, 1993). In addition to N, potato crops also require an adequate supply of micro-nutrients; especially zinc and boron during its growing period. The average potato yield in Pakistan ranges from 20 to 22 tons per hectare, which is far less than 70 to 80 tons per hectare for table potatoes from countries like New Zealand or North America.

Urea is the predominant form of N fertilizer used in Pakistan and worldwide (IFA 2011), mainly because of its high N content (46%) and lower cost per unit N compared to other N fertilizers. However, N recovery by plant from applied urea is always less (<50%) because of the heavy N losses of up to 30% of the applied N as ammonia (NH3) volatilization (Sanz-Cobena *et al.*, 2011). Such high NH_3 losses from applied urea lower the fertilizer use efficiency of applied urea, and thus represent both economic and agronomic losses. In addition NH_3 losses also pose a potential environmental hazard through eutrophication of lakes, rivers and other waterways when NH_3 is re-deposited in these ecosystems. Hence, improving fertilizer use efficiency of urea is therefore critical to minimize farm costs, increase crop productivity and improve livelihood of growers.

Available options to improve fertilizer efficiency of urea include coating urea with some polymers and applying little N more often to better match plant N demand with available N, and applying irrigation water soon after urea application to wash the applied urea from surface soil to minimize the risk of NH_3 volatilization (Black *et al*., 1987; Zaman *et al*., 2013a). Among these options, coating urea with a urease inhibitor (UI) such as N-(n-butyl) phosphorothioic triamide (nBTPT), trade name "Agrotain", to slow urea hydrolysis has the most potential. A number of field and glasshouse trials have reported significant improvement in fertilizer use efficiency after applying granular urea with Agrotain (Watson *et al*., 2008; Zaman *et al.*, 2008; Zaman *et al*., 2010). Among various natural and synthetic UIs, nBTPT is widely used, because it remains effective at a very low concentration (0.025 to 0.1%), and is relatively stable after coating or impregnating urea fertilizer (Watson *et al*., 2008).

Abiotic factors including stress such as water deficiency, increased temperature, strong windy conditions, and exposure to ultra-violet radiation are reported to have negative effects on potato growth and productivity. Researchers have reported that potato yield and quality can be increased after applying different rates of urea (Mahmood *et al*. 2006, Kumar *et al.* 2007). However, no published information is available on the effect of urea, in combination with Agrotain and Cytozyme products, on potato productivity and quality in the

sub-tropical climate. Therefore, the objective of this study was to investigate the agronomic efficiency of different rates of urea applied with nBTPT on potato yield and quality and whether Cytozyme products (Crop+, Bio-Nutrient Combination, and Bio-Nutrient Potassium), which are reported to reduce abiotic stress in crops have any effect on improving the fertilizer use efficiency of applied urea.

2. Materials and Methods

The field trial was conducted on Research Farm, Faculty of Agriculture, Gomal University Dera Ismail Khan (GPS: 31˚ 42'48.80'' N, 70˚ 50' 09.53'' E) K.P.K, Pakistan during 2010-2011. Forty-five field-plots, each 5x3 m^2 and separated by a 1 m buffer zone, were established in five rows. Prior to treatment application, three composite soil samples from 0-10 cm depth were taken for chemical analyses. The soil pH was 7.63. It contained NO_3-N at 0.36 mg kg^{-1}, total P at 2.98 mg kg^{-1}, K at 244 mg kg^{-1}, and ECe was at 1.42 d Sm^{-1}. All plots received a basal rate of 150 kg P ha^{-1} and 200 kg K ha^{-1} using triple superphosphate and sulphate of potash respectively. Tubers were obtained from Hazara Research station, Abbotabad. Cut pieces of potato tubers, each about 40-50 g with at least two eyes, were planted with plant-to-plant spacing of 30 cm and row-to-row of 75 cm on 24 December 2010.

Nine treatments were tested: control (no N or Cytozyme), urea at 200 kg N ha^{-1}, urea at 300 kg N ha^{-1}, Agrotain treated-urea at 200 kg N ha^{-1}, Agrotain treated-urea at 300 kg N ha^{-1}, urea at 200 kg N ha^{-1}+Cytozyme, urea at 300 kg N ha^{-1}+Cytozyme, Agrotain treated-urea at 200 kg N ha^{-1}+Cytozyme, and Agrotain treated-urea at 300 kg N ha^{-1}+Cytozyme. Each treatment was replicated five times. The Cytozyme tested products included Seed+, Crop+, Bio-Nutrient Combination, and Bio-Nutrient Potassium. Seed+ was applied to the seeds at 2 kg/ton seed at the time of planting, while Crop+, Bio-Nutrient Combination and Bio-Nutrient Potassium were applied at 2 L/ha each at timing of fertilizer application. Urea with or without Agrotain was applied in three split doses, first dose at 30 days after planting, second at 20 days after first application and third at 30 days after the second application. The Cytozyme products (Crop+, Bio-Nutrient Combination, and Bio-Nutrient Potassium) were also applied with the three split applications of N. The experiment was conducted in a randomized complete block design. Common farming practices in the region like spraying herbicides and insecticides and irrigation were also followed. Growth and yield parameters included survival percentage, plant height, plant spread or canopy area, number of stems per plant, total yield, and yield and number of large, medium and small tubers. Potato crops were harvested in the first week of May after reaching maturity. Tubers from each plot were dug out by hand, brushed to remove loose soil, counted, separated into large (>55 mm), medium (35-55 mm) and small (<35 mm) grades according to their sizes. Each grade tubers were weighed for calculating tuber yield.

2.1. Statistical Analysis

Analysis of variance (ANOVA) was performed to compare fertilizer treatments with respect to various measured parameters. When significant effects of treatment were observed, these were further explored using the Tukey adjusted LSD value to make specific comparison among the different treatments. All the analyses were performed using Minitab (version 12).

3. Results and Discussion

3.1. Effect of applying urea with Cytozyme or Agrotain on Plant growth parameters

Compared to the urea-alone treatment, urea applied with Cytozyme or with Agrotain had a positive effect on plant survival percentage, plant height, plant canopy or spread and number of stems per plant (Table 1). Plant survival percentage was significantly higher from treatments receiving Agrotain-treated urea plus

Cytozyme than in the urea-only treatments. The highest survival rate was recorded from treatments with Agrotain-treated-urea-200+Cytozyme (91.4%), followed by urea-200+Cytozyme (89.6%) and urea-300+Cytozyme (88.7%). This represents an increase in survival percentage by 4.2, 4.2 and 2.4%, respectively, over their corresponding treatments without Cytozyme. With urea application at 200 kg N ha^{-1}, potato plants were significantly taller with Cytozyme treatments than those receiving only urea, or Agrotain-treated urea (Table 1). Agrotain-treated urea applied at 200 kg N ha^{-1} with Cytozyme resultedin a mean plant height of 47.95 cm, an increase of 9% over the corresponding treatment without Cytozyme. Compared to the treatment without Cytozyme, plant height was 12% and 7% greater following the Cytozyme application with commercial urea at 200 and 300 kg N ha^{-1}, respectively. Plant spread was also significantly (p<0.05) higher in potato crops that received Cytozyme, either with Agrotain-treated urea or with urea alone, compared to the corresponding treatments without Cytozyme (Table 1). The maximum plant canopy widths were 61.3, 60.8, and 60 cm for potato plants receiving Agrotain treated-urea-200+Cytozyme, Agrotain treated urea-300+Cytozyme, and urea-300+Cytozyme, respectively. This represents a 9.8, 7.9, and 8.6% increase, respectively, relative to the corresponding treatments without Cytozyme. The number of stems per plant was also significantly (p<0.05) higher for applied urea with Cytozyme or Agrotain, at 200 kg N ha^{-1} (Table 1).

Table 1. Effect of urea applied at two rates (200 and 300 kg N ha^{-1}) with or without urease inhibitor (Agrotain) and Cytozymes on plant survival, plant height, plant spread and number of stem per plant of potato in a field trial. Values are means (n=5).

Treatments	**Plant survival (%)**	**Plant height (cm)**	**Plant Spread (cm)**	**Stem/ plant**
Control (no N or Cytozyme)	84.4 f	38.07 f	42.03 e	2.64 c
Urea-200	86. e	40.97 e	54.43 d	3.44 b
Urea-300	86.6 e	42.63 de	55.24 cd	3.52 b
Agrotain treated-urea-200	87.7 d	44.07 cd	55.80 cd	3.44 b
Agrotain treated-urea-300	89.1 bc	45.94 b	56.4 c	3.44 b
Urea-200 + Cytozyme	89.6 b	45.83 b	59.14 b	3.64 b
Urea-300 + Cytozyme	88.7 c	45.76 bc	60.02 ab	3.56 b
Agrotain treated-urea-200 + Cytozyme	91.4 a	47.95 a	61.32 a	3.91 a
Agrotain treated-urea-300 + Cytozyme	87.7 d	46.47 ab	60.86 a	3.56 b
LSD	0.86	1.71	1.41	0.27

These significant improvements in potato growth parameters are related to the positive effect of applying urea with either Cytozyme or with urease inhibitor (UI) "Agrotain". On a daily basis, all plants experience positive (water, light, CO_2, O_2, fertilizer) and negative (drought, heat, cold, disease, flooding) stress (signals) and Cytozyme is known to alleviate these abiotic stress. For each of these signals there are membrane-bound receptors (in plasma lemma and membranes of cell organelles) that are responsible for interpreting the signals. The biochemical pathway can either be part of primary or secondary metabolism. When the sun rises in the morning, the plant reacts to the light signal by activating the primary metabolic process photosynthesis,while the opposite occurs when the sun sets in the evening. In contrast, the primary metabolic process of respiration is not light dependant, but oxygen (O_2) serves as the signal to activate the process 24 h per day. If a plant is resistant towards drought, the drought signal will activate specific genes that will lead to increased abscissic acid (ABA) and proline levels (both are secondary metabolites) via the mevalonic acid pathway and glutamate shunt respectively. Both proline and ABA are involved in drought resistance in plants. It is postulated that the plant extracts and nutrients in Cytozyme include compounds that stimulate plant growth, improve physiological functions and reinforce plant defence mechanisms against abiotic and biotic stress factors. Improved plant growth parameters like better canopy and increased height play a significant role in the final yield of a crop, especially in case of tuber crops (Rahman *et al.* 2004 ; Mahmood *et al*. 2006), which will be discussed in below section.

3.2. Effect of applying urea with Cytozyme or with Agrotain on tubers yield and quality

Urea applied with Cytozyme or with Agrotain had a significant (p<0.05) influence on potato total tuber yield and grades (large, medium and small) (Table 2). Cytozyme treatments were more effective when applied with urea, than applied with Agrotain-treated urea. Better response from applied urea with or without UI was observed at 200 kg N ha^{-1} than at 300 kg N ha^{-1}. Cytozyme application with urea increased the tuber yield by 53% and 35% for urea at 200 kg N ha^{-1} and urea at 300 kg N ha^{-1} respectively, compared to the equivalent urea-only treatments. Such improvement in total tuber yield by Cytozyme with urea fertilizer could be attributed to the combined effect of N supply from applied urea and reduced abiotic stress by Cytozyme. Improvement in tuber yield were lacking when Cytozyme was applied with Agrotain-treated urea at the higher rate (300 kg N ha^{-1}). Such depression in yield at the higher rates (300 kg N ha^{-1}) indicate a negative interaction between Cytozyme and Agrotain treated urea which could be due to some unknown chemical reaction between nBTPT, N and Cytozyme constituents and need further investigation. However, no such negative interaction was seen when Cytozyme was applied with Agrotain treated urea at 200 kg N ha^{-1}. For example Cytozyme applied with Agrotain treated urea at 200 kg N ha^{-1} produced 14% more total tuber yield compared to its corresponding rate without Cytozyme. Total tuber yield were also significantly higher when urea was applied with urease inhibitor Agrotain. Agrotain-treated urea applied at 200 kg N ha^{-1} produced 25,450 kg ha^{-1} of tubers compared to 17,428 kg $ha^{-1}$from the equivalent urea-only treatment, thus reprenting an increase of 46%. Similarly, Agrotain-treated urea at 300 kg N ha^{-1} produced a tuber yield of 26,852 kg ha^{-1} compared to 18,867 kg ha^{-1} an increase of 42% from the equivalent urea-only treatment. The yield of large-sized potato tubers was significantly (p<0.001) higher in crops receiving Agrotain-treated urea than standard urea treatments (Table 2). Agrotain-treated urea at 200 kg N ha^{-1} produced 11,724 kg ha^{-1} of large-sized tubers compared to 10,012 kg ha^{-1} with urea alone at the same N rate, representing an increase of 17% over urea alone. Similarly, Agrotain-treated urea at 300 kg N ha^{-1} produced a large-sized tuber yield of 15,208 kg ha^{-1} compared to 9,516 kg ha^{-1} with urea alone at 300 kg N ha^{-1}, a 60% increase. We attribute the high fertilizer use efficiency of urea treatments containing nBTPT in our research to the interaction of four chemical and biochemical reactions which appear

to be facilitated by nBTPT in the presence of urea. These reactions include (i) delayed urea hydrolysis which reduces ammonia losses (Zaman *et al.*, 2008, 2009, 2013b; Zaman and Nguyen 2012), (ii) additional time for increased lateral and downward movement of urea in the soil (Dawar *et al*., 2011), (iii) reduced nitrification (Sanz-Cobena *et al*. 2012), and (iv) more efficient N uptake and conversion into plant protein (Castle *et al.*, 2006; Dawar *et al*., 2012, Zaman *et al*., 2013b). The positive effective of applying urea with Cytozyme are related to alleviating the abiotic stress and better N nutrition as already discussed above. The benefits achieved by using Agrotain-treated urea compared to urea only in term of total tuber yield improvements were the same for both lower (200 kg N ha^{-1}) (46%) and higher rate (300 kg N ha^{-1}) probably because Agrotain only delays urea hydrolysis by 1 to 2 weeks during which time plants have access to N take in urea form. It is possible that at 200 kg N ha^{-1}, N uptake is maximized in that 1 to 2 weeks period so there is no extra advantage over urea achieved by applying more Agrotain treated urea at the 300 kg N ha^{-1} application rate. When applied with urea, Cytozyme increased the tuber yield by 61% and 62% compared to the equivalent treatments with urea at 200 kg N ha^{-1} and urea at 300 kg N ha^{-1}, respectively.

Table 2. Effect of urea applied at two rates (200 and 300 kg N ha^{-1}) with or without urease inhibitor (Agrotain) and Cytozymes on tuber yield of all sizes. Values are means (n=5).

Treatments	**Tuber yield (kg/ha) of different sizes**			
	All tubers	**Large (>55 mm)**	**Medium (35-55 mm)**	**Small (<35 mm)**
Control (no N or Cytozyme)	12,948	7108	3956	2290
Urea-200	17,428	10,012	5088	2328
Urea-300	18,867	9516	5330	4019
Agrotain treated-urea-200	25,450	11,724	8046	5680
Agrotain treated-urea-300	26,852	15,208	6996	4648
Urea-200 + Cytozyme	26,676	16,084	7046	3546
Urea-300 + Cytozyme	25,972	16,528	6134	3310
Agrotain treated-urea-200 + Cytozyme	28,964	16,746	8156	4242
Agrotain treated-urea-300 + Cytozyme	25,452	15,448	6595	3409
LSD	**4280**	**3333**	**2033**	**1582**

In crops treated with Agrotain-treated urea at 200 kg N ha^{-1}, Cytozyme application increased the large-sized tuber yield to 16,746 kg ha^{-1} compared to 11,724 kg ha^{-1} without Cytozyme, representing an increase of 43%; however, no such trend was observed when Cytozyme was applied with Agrotain-treated urea at 300 kg N ha^{-1}. Yields of medium- and small sized potato tubers followed a similar trend and were significantly (p<0.001) higher in treatment receiving urea with Cytozyme or urea with Agrotain-compared to plots receiving urea alone (Table 2, 3). Overall, large- and medium-sized potato tubers each comprised 30% of the total tubers, with small tubers being ≥ 40% of the total (Table 3).

Table 3. Effect of urea applied at two rates (200 and 300 kg N ha^{-1}) with or without urease inhibitor (Agrotain) and Cytozymes on percentage of tubers sizes (large, medium and small) in a field trial. Values are means (n=5).

Treatments	**Percentage of tuber sizes**		
	Large (>55 mm)	**Medium (35-55 mm)**	**Small (<35 mm)**
Control (no N or Cytozyme)	28	30	43
Urea-200	29	31	40
Urea-300	26	27	47
Agrotain treated-urea-200	22	30	48
Agrotain treated-urea-300	28	30	42
Urea-200 + Cytozyme	31	30	38
Urea-300 + Cytozyme	33	26	42
Agrotain treated-urea-200 + Cytozyme	28	31	42
Agrotain treated-urea-300 + Cytozyme	31	30	39

4. Conclusions

This field study has demonstrated that it is possible to achieve a significant improvement in potato productivity and quality by applying urea with either Agrotain or with Cytozyme. Agrotain appears to improve the bioavailability of the applied urea and thus resulted in better improvement in potato yield and quality at the lower rate (200 kg N ha^{-1}). Cytozyme improved tuber yield and quality when it was applied with standard urea; applying it with Agrotain-treated urea resulted in a yield depression at the higher rate (300 kg N ha^{-1}). Further research is required to investigate the interaction between Agrotain (nBTPT) and nutrients and chemicals in Cytozyme.

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