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EFFECT OF CADMIUM ON GERMINATION AND GROWTH OF WHEAT

ABSTRACT: In this study, the effect of Cd on the germination, growth of seedlings and composition of plants deriving from contaminated grains, grown in the field, was examined. Wheat grains were soaked in Cd-containing solutions: 0 (control, deionized water), 10^{-5}, 10^{-4}, 10^{-3}, and 10^{-2} M $CdCl_2$ during 24h. One portion of grains was used to test germination and seedling growth, while the other was sown in the field. The concentration of Cd in the grains almost linearly increased with the increase in the applied concentrations of Cd, which reduced the germination and energy of germination and increased the proportion of atypical seedlings. Larger concentrations of Cd significantly impaired the growth of seedlings (length of the shoots and roots, dry matter mass). In the grains of the field-grown plants the concentrations of N, P, and K were not affected by Cd, but their concentrations in the straw declined (especially of N). These results suggest that the emergence and development of plants on the soil polluted by Cd are likely to be significantly limited and yield reduced.
KEYWORDS: cadmium, concentration of N, P, and K, germination, grain, growth, straw, wheat

INTRODUCTION

Cadmium (Cd) belongs to a group of heavy metals without possessing a known biologically beneficial role. It is present in low concentrations in nature. Only in the era of industrial revolution, Cd became an important pollutant of the environment (soil, water, and air). Cadmium is equally toxic for living organisms – plants, animals, and humans (Gupta and Gupta, 1998). It jeopardizes key physiological processes in plants already at low concentrations (Kastori et al.,

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1997, Benavides et al., 2005). It affects the activity of enzymes (Kuriakose and Prasad, 2008), inhibits photosynthesis (Panković et al., 2000), induces oxidative stress (Ćosić et al., 2018), affects the uptake, accumulation and translocation of mineral elements (Jiang et al., 2004, Maksimović et al., 2007), affects water regime (Barceló et al., 1986), as well as plant anatomy (Maksimović et al., 2007; Luković et al., 2014).

Germination is the initial and crucial phase in the life cycle of higher plants. It is a very complex process (Mayer and Poljakoff-Mayber, 1989) during which significant physiological, biochemical and morphological transformations take place. Therefore, qualitative and quantitative changes induced by the action of external factors during the course of germination may have a negative effect on germination and sprouting. Having in mind that its phytotoxicity and the fact that Cd is a significant potential pollutant of agricultural soils, the aim of this study was to explore the effect of the increasing concentrations of Cd on the germination and growth of wheat seedlings under laboratory conditions, as well as on the concentration of nitrogen, phosphorus, and potassium in the grains and straw of next generation in the field.

MATERIAL AND METHODS

Winter wheat variety Pobeda was used in the experiments. It has been previously found that wheat grain soaked during 24h at 26 °C contains approximately 38% of water and after that period there is no statistically significant increase in water content in the seed. Wheat grains need about 30% of water to germinate. Therefore, during this experiment, Cd treatment was performed by soaking the wheat grains in water solutions of $CdCl_2$ at the following concentrations: 0 (control, deionized water), 10^{-5}, 10^{-4}, 10^{-3}, and 10^{-2} M Cd during 24h and then rinsed with deionized water. The effect of treatments was examined in the laboratory and in the field.

Examination of the effects of Cd on germination comprised the analyses of germination energy, germination percent, the portion of atypical seedlings and ungerminated grains, according to ISTA 2011 protocols. Germination took place on the filter paper, at 20 °C; germination energy was recorded on the fourth day after sowing and germination on the eighth day after sowing. Seedlings without root and with undeveloped coleoptiles were declared as atypical. The experiment was done in five replications.

The effect of Cd on the growth of seedlings was established on the tenth day after sowing, in the laboratory, at around 23 °C. The following parameters were recorded: length of shoots and roots, fresh weight and dry weight (after drying of plant material at 80 °C to constant mass). All measurements were taken in five replications, with 10 seedlings per replication.

To examine the effect of the imbibitions of wheat grains in the solutions containing Cd on the concentration of N, P, and K in the grains and straw of the progeny of imbibed grains, the other portion of imbibed grains were sown in the field, on the soil classified as a calcic, gleyic chernozem (Loamic, Pachic-CH-cc.gl-Ip. ph [IUSS Working Group WRB, 2015]), of weak alkaline reaction, medium humus content, and optimal concentrations of N, P, and K. The concentration of Cd in the soil was significantly lower than the maximally allowed (2 mg/kg soil) and lower also than the usual concentration of Cd in the soil (0.2 to 1 mg/kg soil). In the topsoil layer (0 to 20 cm), where Cd accumulates the most, concentration in EDTA extracted fraction was 0.206, and a total content was 0.703 mg/kg of the soil. During the experiment, the usual management practices for wheat production were applied.

The concentration of N in the grains and straw of wheat was assessed by micro Kjeldahl method, of P spectrophotometrically using the vanadate-molybdate method, and of K by flame photometry. The concentration of Cd in grains was measured by inductively coupled plasma emission spectrometer, after digestion of grain wholemeal in a mixture of 10 ml HNO_3 (65%) and 2 ml H_2O_2 (30%) using microwave technique.

Statistical analyses were done by Statistica, version 13.3.

RESULTS AND DISCUSSION

Concentration of Cd in the grains imbibed in the solutions containing Cd increased almost linearly with an increase in the concentration of Cd in the solution, whereas concentration of Cd in the grains imbibed in the deionized water without Cd (control) was 0.032 mg/kg of dry mass (Figure 1), which

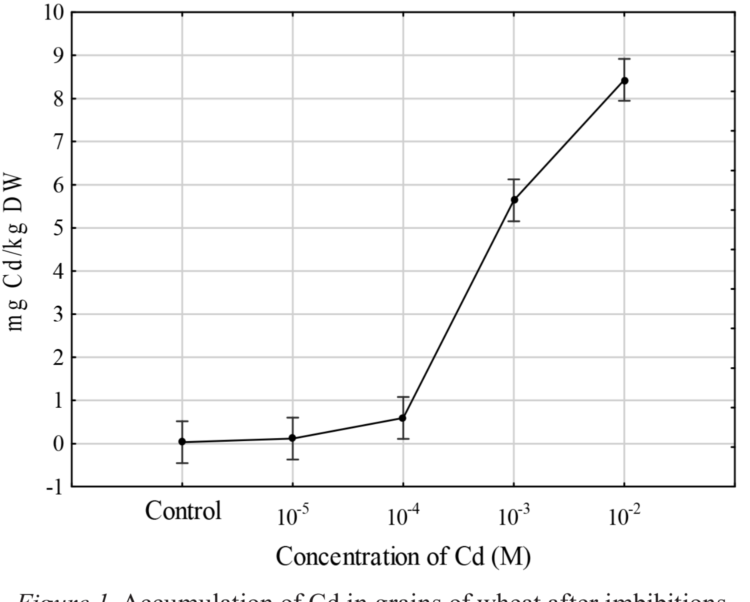

Figure 1. Accumulation of Cd in grains of wheat after imbibitions in solutions containing Cd

corresponds to the average concentration of Cd in uncontaminated grains of wheat (Kabata-Pendias, 2000). Rinsing of grains after exposure to the solutions containing Cd with deionized water served to eliminate Cd which was on the surface of the grain, attached to bran. According to Kuriakose and Prasad (2008), with an increase in the concentration of Cd the absorbed amount of Cd increases. Inhibitory effect of Cd on the germination of various plant species was recorded by a number of authors (Mrozek, 1980; Naquib et al., 1982; Rani et al., 1990; Chugh and Sawhney, 1996; Pandit and Prasannakumar, 1999; Kuriakose and Prasad, 2008) and it is in accordance with our results obtained in wheat. With an increase in the concentration of Cd in the imbibition solution, germination and energy of germination declined (Figure 2).

Germination in wheat commences with the absorption of water. The first phase in this absorption is dependent on the colloid system of the grains. Hydrophilic groups attract dipole molecules of water. By activation of hydrolytic enzymes, large organic molecules are hydrolyzed and the concentration of osmotically active molecules in the grain increase, which allows intensive absorption of water by the grains. Kuriakos and Prasad (2008) found that the content of water was significantly lower in the grains exposed to Cd. This leads to a conclusion that Cd reduces the uptake of water by the grains during germination, most probably in the phase in which absorption of water is directly dependent on the presence of osmotically active compounds. Their presence, however, depends on the activity of hydrolytic enzymes.

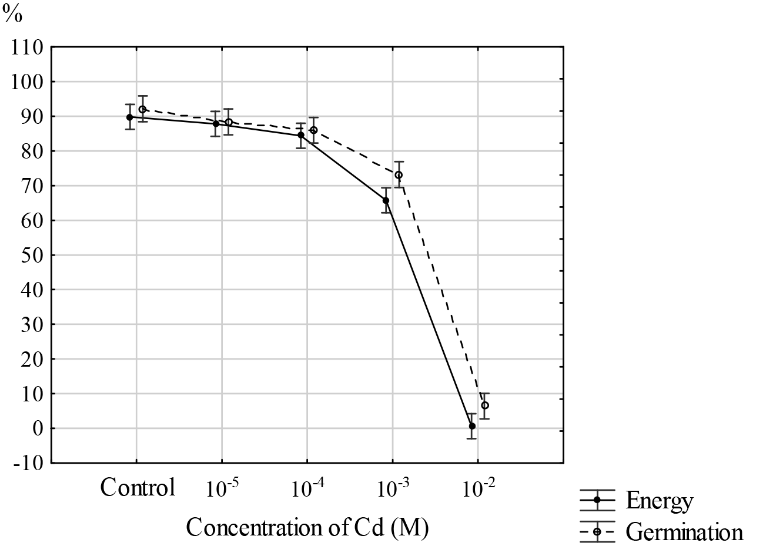

Figure 2. The energy of germination and germination after imbibition of wheat grains in solutions containing Cd

It was found that in seeds of pea exposed to the increasing concentrations of Cd during germination, total amylolytic activity and activities of α- and β- amylases, as well as respiration rate, declined (Chugh et al., 1992). Kuriakose and Prasad (2008) found that activities of acid phosphatase, protease, and α-amylase declined during germination of sorghum seeds exposed to the increasing concentrations of Cd. It is considered that Cd reduces not only the decomposition of insoluble sugars but also the transport of soluble sugars to the embryo, which is an important precondition for the development of the embryo and therefore for germination. Processes of decomposition of the organic compounds stored in the endosperm are controlled by phytohormones (Mayer and Poljakoff-Mayber, 1989). During germination, in the coleoptile and scutellum synthesis of gibberellins takes place. Gibberellins then diffuse to aleurone cell layer, where they induce synthesis and activation of hydrolytic enzymes. Hydrolytic enzymes are then released from aleuronic cell layer to endosperm. Besides α-amylase, gibberellic acid induces the formation of the other enzymes in aleuronic cell layer, such as proteases, carboxypeptidase, ribonuclease, arabinofuranozidaze, and acidic phosphatase (Jones and Jacobsen, 1991). Alpha-amylase is a key enzyme of the entire process of the mobilization of nutrients from the endosperm. In wheat grains, α-amylase, which commences the hydrolysis of starch, is synthesized *de novo* during germination, whereas β-amylase is present in the starchy endosperm in either free or bound state (Gallaeschi and Chapman, 1985). Gibberellic acid stimulates transcription and translation steps in the process of synthesis of α-amylase during germination (Ökkes et al., 2003). According to Maksimović et al. (2018) plant height, the number of spikes per m^2 and grain yield in wheat at harvest significantly declined with the increase in Cd concentration from 0 to 10^{-5} M in the solution in which the grains were imbibed prior to sowing. The height of plants deriving from grains treated with the highest concentration of Cd was reduced by 25%. This suggests that Cd may have reduced the activity of gibberellic acid and in this way the elongation of stems – in other words, Cd probably had a direct impact on gibberellic acid. This presumption is aided by the results of Ökkes et al. (2003) who found that Cd inhibited the activity of α-amylase and synthesis of gibberellic acid during pea seed germination. Details on the mechanism of the inhibition of synthesis of gibberellic acid remain to be further elucidated. The fact that the application of gibberellic acid reduces stress induced by Cd highlights the importance of the interaction between Cd and gibberellic acid (Ghorbanli et al., 1999; Hadi et al., 2014).

Besides the reduction in germination and energy of germination, with the increase in concentrations of Cd in the solution in which grains were imbibed the portion of atypical seedlings and ungerminated grains also increased (Figure 3), which confirms the complexity of the impact of Cd on plant development.

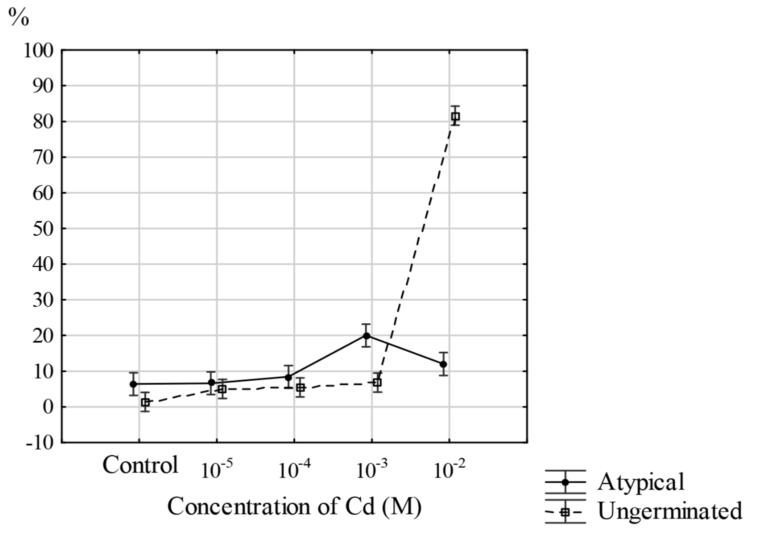

Figure 3. The portion of atypical seedlings and ungerminated grains after imbibition of grains in solutions containing Cd

Higher concentrations of Cd significantly reduced the length of shoots and roots (Figure 4), as well as their dry mass (Figure 5). The growth of shoots and roots was reduced more than dry mass, suggesting that Cd affected growth factors more severely than the accumulation of dry weight. Lux et al. (2011) showed that the presence of Cd in the rhizosphere inhibits elongation of roots and affects their anatomy.

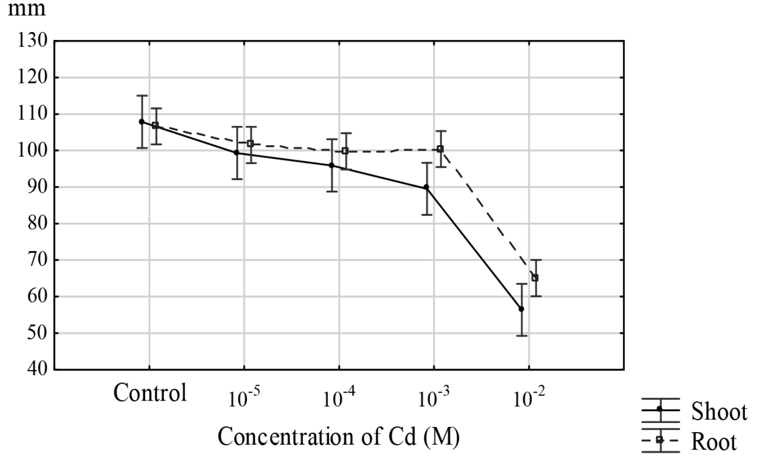

Figure 4. Length of shoots and roots of wheat seedlings exposed to Cd during germination

They also suggest that the concentration of Cd is most often higher in roots than in shoots because of restricted transport of Cd through xylem in the majority of analyzed plant species.

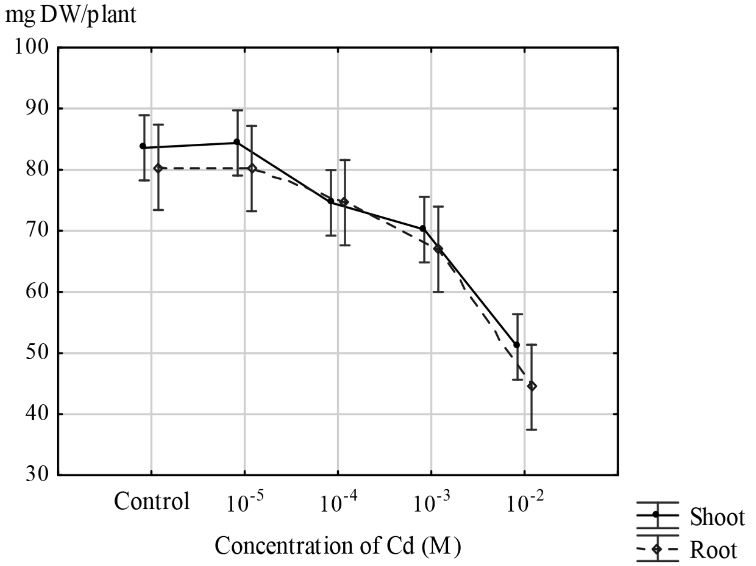

Figure 5. Dry mass of shoots and roots of seedlings exposed to Cd during germination and measured 10 days after sowing

Seedlings deriving from seeds exposed to the highest concentration of Cd were less hydrated that unexposed. The water content in the shoots was around 4% lower. Maksimović et al. (2007) observed significant changes in root anatomy of maize seedlings treated with Cd, which included also thickening of the cortex, and this may be the reason for the increased resistance to lateral transport of water from root surface to xylem vessels and therefore reduced water uptake. They also found that plants exposed to Cd were less hydrated. Kastori et al. (1992) found that sunflower plants exposed to Cd reduced transpiration intensity when compared to the control. All these findings lead to a conclusion that the toxic concentrations of Cd may affect the metabolism of young plants partly due to insufficient water provision, apart from the reduced transpiration. However, it is important to stress that the increase in the concentration of free proline, which is a reaction typical for water stress, occurs also in the presence of Cd in turgid plants (Kastori et al., 1992).

Higher concentrations of Cd may affect the accumulation and distribution of mineral elements in plant tissues (Maksimović el al., 2007; Putnik-Delić, 2013). Exposure of grains to the increasing concentrations of Cd, however, did not change the concentration of N, P, and K in the grains of the next generation (Figure 6), but it reduced the concentration of these elements, especially N, in the stems at physiological maturity (Figure 7). Damages of the root system of young plants due to higher concentrations of Cd may have an unfavorable effect later, during vegetative growth (Chugh and Sawhney, 1996).

Cadmium is considered to influence nitrogen metabolism, but the effect may be direct and/or indirect (Kastori et al., 1997). Muhammad et al. (2008)

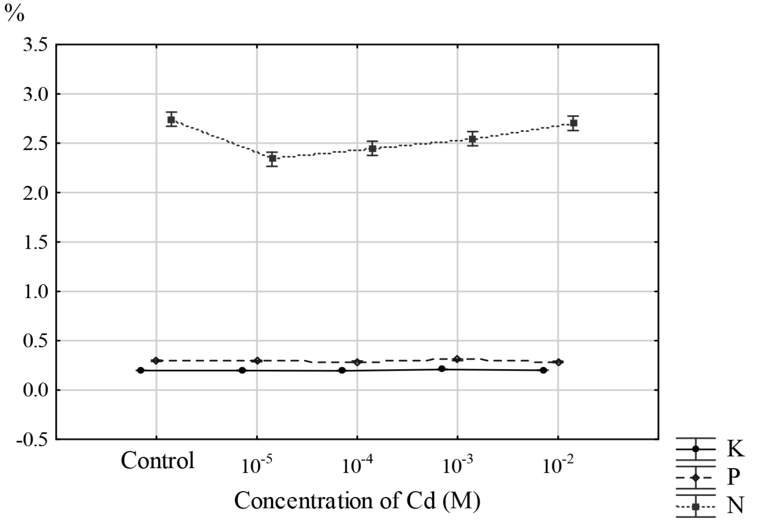

Figure 6. The concentration of N, P, and K in the grains of wheat which are the progeny of grains exposed to Cd during germination

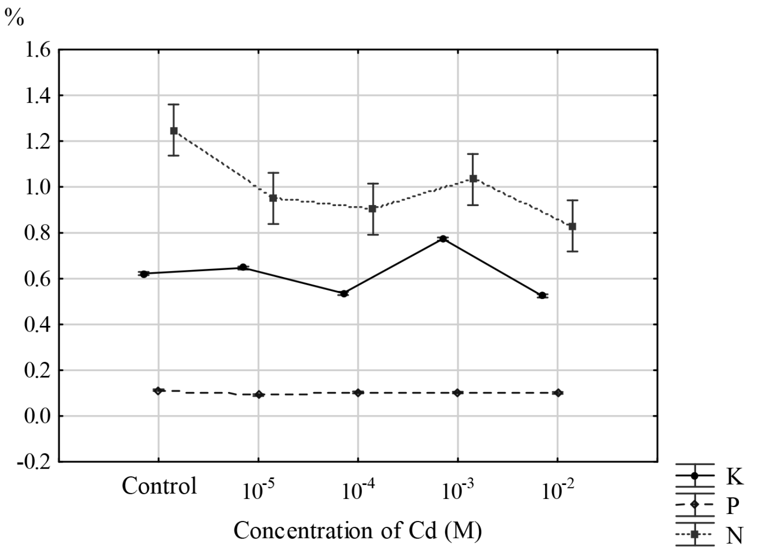

Figure 7. The concentration of N, P, and K in the straw of wheat which was derived from grains exposed to Cd during germination

found that Cd significantly reduced the concentration of N in roots and especially in stems of rice, as well as the activity of nitrate reductase. Similar changes in the activity of nitrate reductase, due to excessive concentrations of Cd, were also shown by Petrović et al. (1991). In addition, Panković et al. (2000) showed that optimal nitrogen nutrition reduces the unfavorable effects of Cd on the photosynthesis of sunflower.

All these results represent evidence of the complex interactions between Cd and plant nutrients.

CONCLUSION

Swelling of wheat grains, imbibed in solutions containing the increasing concentrations of Cd, nearly linearly increased the concentration of Cd in those grains and significantly reduced germination and energy of germination, and concomitantly increased the proportion of atypical seedlings. Higher concentrations of Cd significantly reduced the growth of seedling. The growth of shoots and roots was reduced more than the dry mass, suggesting that Cd affected the growth factors to a greater extent than dry biomass production. The highest applied concentration reduced the hydration of shoots. In the field, in the grains of plants which are the progeny of grains exposed to Cd, concentrations of N, P, and K were not changed but in the straw, at maturity, their concentration (especially concentration of N) was lower than in the control. These results suggest that germination is very sensitive to the presence of higher concentrations of Cd.

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УТИЦАЈ КАДМИЈУМА НА КЛИЈАЊЕ СЕМЕНА И РАСТ ПШЕНИЦЕ

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РЕЗИМЕ: Кадмијум (Cd) је потенцијални загађивач средине живих организама и при већим концентрацијама одликује се јаким фитотоксичним дејством. У раду је у лабораторијским условима проучено дејство различитих концентрација кадмијума на клијање и раст младих биљака пшенице, а у пољским условима његов утицај на концентрацију минералних материја у зрну и слами у фази пуне зрелости биљака, које су потекле од зрна третираног кадмијумом. С повећањем концентрације кадмијума у фази бубрења семена скоро се линерано повећала његова концентрација у семену. Утврђено је да веће концентрације кадмијума значајно смањује клијање и енергију клијања семена, а повећавају учешће атипичних клијанаца и непроклијалог семена. Веће концентрације кадмијума значајно су смањиле раст младих биљака као и масу њихове суве материје. Раст надземног дела и корена смањен је у већој мери од масе суве материје, на основу чега се може закључити да је кадмијум у већој мери утицао на факторе раста него на стварање суве масе. Највећа примењена концентрација кадмијума смањила је хидратисаност надземног дела младих биљака. Бубрење семена у присуству различитих концентрације кадмијума није утицало на концентрацију азота, фосфора и калијума у зрну, а смањило је њихово присуство у слами, нарочито азота на крају вегетације. Добијени разултати потврђују налазе ранијих истраживања код других биљних врста на основу којих су физиолошки и биохемијски процеси клијања семена значајно инхибирани у присуству већих концентрација кадмијума. На основу наведеног може се предвидети да ће на земљишту загађеном кадмијумом ницање усева и раст младих биљака пшенице бити ограничено, што ће се неповољно одразити на принос усева.

КЉУЧНЕ РЕЧИ: кадмијум (Cd), клијање, концентрације азота (N), фосфора (P) и калијума (K), пшеница, раст, слама, зрно