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NATURAL OCCURENCE OF DEOXYNIVALENOL AND OCHRATOXIN A IN CONVENTIONAL MAIZE HYBRIDS AND THEIR BIOSAFETY COMPARED WITH GM EQUIVALENTS

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ABSTRACT. Familiarity based approval of the newly developed GM cereal events is based upon the stable and safe consumption of conventional grains. The level of concentrations of mycotoxins and biomolecules establishes the criteria for pre-market evaluation of genetically modified cereals e.g. MON 810 maize. The objective of the present study was to comparatively evaluate food biosafety of the conventional and GM maize. Grain samples from the harvest lot of 10 maize hybrids in the year 2011 were collected arbitrarily. Well ground and homogenized samples were analysed for the deoxynivalenol (DON) and ochratoxin A (OTA) mycotoxins. Contamination rates and levels of DON and OTA were low and did not exceed the maximum levels, indicating their possible safe use as food and feed under the EC regulation 1881/2006.The samples were further analysed for the possible effect of mycotoxin concentration upon that of starch and proteins. The study reveals the absence of any negative impact of the presence of mycotoxins upon these biomolecules as their concentrations lie within the normal range. A comparative review of data for the mycotoxins in conventional maize grains invalidate the argument from the producers of GM maize hybrids that conventional hybrids are inferior for food biosafety with respect to mycotoxins.

Key words: Conventional maize; Deoxynivalenol; Ochratoxin A; Food biosafety.

REZUMAT. Frecvența naturală a deoxinivalenolului şi a ochratoxinei A în hibrizii convenționali de porumb şi biosecuritatea acestora, comparativ cu echivalente modificate genetic. Aprobarea apariției de noi cereale modificate genetic (MG) pe baza conceptului de familiaritate se bazează pe consumul stabil şi sigur al cerealelor convenționale. Nivelul concentrațiilor de micotoxine şi biomolecule stabileşte criteriile de evaluare a cerealelor modificate genetic înainte de comercializarea acestora, de exemplu, apariția porumbului MON 810. Obiectivul prezentului studiul a fost de a evalua, comparativ, biosecuritatea alimentară a porumbului convențional şi a celui modificat genetic. Probele din lotul de 10

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hibrizi de porumb din anul 2011 au fost colectate arbitrar. Probele, bine macinate şi omogenizate, au fost analizate pentru a se evalua conţinutul de micotoxine, deoxinivalenol (DON) şi ochratoxina A (OTA). Ratele de contaminare şi nivelurile de DON şi OTA au fost reduse şi nu au depăşit nivelurile maxim admise, ceea ce indică posibilitatea de a fi folosite în siguranţă ca hrană şi nutreţ, în conformitate cu regulamentul EC nr. 1881/2006. De asemenea, probele au fost analizate pentru a determina posibilul efect al concentraţiei de micotoxine asupra concentraţiei de amidon şi proteine. Studiul relevă absenţa vreunui impact negativ al prezenţei micotoxinelor asupra acestor biomolecule, concentraţiile acestora fiind la un nivel normal. O analiză comparativă a datelor privind micotoxinele din boabele de porumb convenţional invalideaza argumentul producatorilor de hibrizi de porumb MG, care susţin că hibrizii convenţionali sunt inferiori, din punct de vedere al micotoxinelor, pentru biosecuritatea alimentară.

Cuvinte cheie: porumb convenţional; deoxinivalenol; ochratoxina A; biosecuritate alimentară.

INTRODUCTION

Depending upon the genetic resistance of maize hybrids, the accumulation of mycotoxins in maize differ significantly. Weather conditions (Reid *et al.,* 1999), genetic variability among the pathogen populations (Carter *et al.,* 2002) and physical traits of the maize hybrids such as husk thickness (Warfield and Davis, 1996) significantly affect the degree of fungal attack. Today, the introduction of new GM IR maize hybrids is pretexed that it is more biosafe than the conventional hybrids (*Fig.1*). The concentrations are expressed in µg/kg. The data does not indicate the specific mycotoxins assessed for contamination levels.

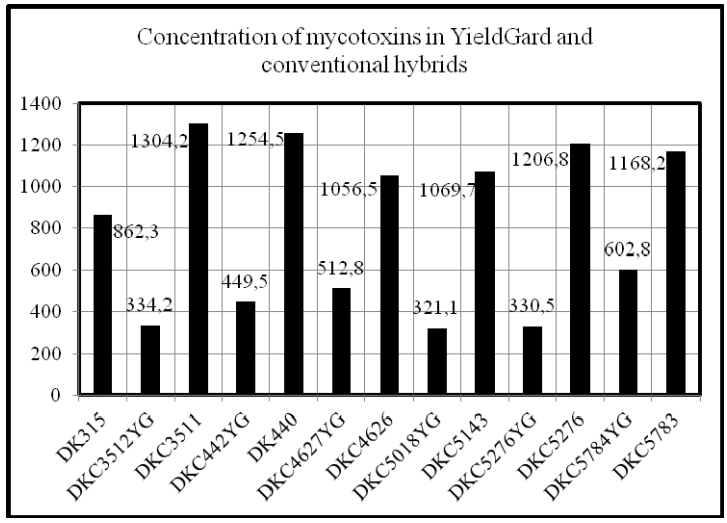

Figure 1 - The comparative difference of cumulative concentration of mycotoxins in YieldGard and conventional maize hybrids. Source: Monsanto (2011)

The primary infection pathway for infection of maize kernels by *Fusarium*, *Aspergillus* and *Penicillium spp.* is via the silks and the spores reach the silks by splashing, wind dispersal or insect vectors. However, the insect injury appears to be a less important pathway than silk infection. Adverse effects of mycotoxin accumulation in cells may lead to a reduction in concentrations of carbohydrates and proteins. The safety of conventional maize hybrids for the levels of specific mycotoxins and concentration effect of these toxins on major cellular biomolecules must be evaluated.

In this experiment, the comparative biosafety of the conventional maize hybrids was analysed for concentration of DON and OTA and their effect on amounts of starch and proteins.

MATERIALS AND METHODS

Samples of conventional maize grains were obtained at the harvest time at the National Agricultural Research and Development Institute Fundulea, Călăraşi County, Romania, in September 2011. All 10 maize hybrids, viz Crişana, Mostiştea, Rapsodia, Milcov, Olt, F-125-06, F-475, F-322, F-254-08 and F-225-06 are commonly grown and adapted to climatic conditions of the area. All the experimental activities were carried out at the laboratories of the Faculty of Biotechnology of University of Agronomic Sciences and Veterinary Medicine Bucharest, Romania.

For DON, 1 kg sample was sieved by applying ¼ method till a laboratory sample of 100g was obtained.The sample was ground. A mixture of 12.5 g/25 ml distilled water was prepared and agitated at 200 rmp/30 min./25 ^{0}C.The filtered elute was centrifuged.After centrifugation and filteration,the elute were extracted through acetonitrile + methanol + water (5:5:90). A 100 ml elute was injected into HPLC apparatus for quantification of the mycotoxin. The limit of detection (LOD) for DON was 0.02 µg/ml.

For OTA, the samples were prepared according to standard procedure. Dilution was carried out with 44 ml tampon phosphate 20 mM pH 7,0 followed by washing through Ochraprep immunoaffinity columns. After the segregation of molecules,100 µl was injected for chromatography. LOD was kept at 0.005 µg /ml. The data for DON and OTA was obtained and worked upon using the software Empower.

The amount of proteins was measured through the standard Kjheldal method while the concentration of starch was measured by Lippich polarimeter by using the expression: Starch %=$\alpha \cdot Vt \cdot 100 / [\alpha]^{20} D.l.p$ where α = measured angle, Vt = sample volume, [α] = standard angle (183.7 for maize), l = tube length (20 cm), p = sample mass (g).

RESULTS AND DISCUSSION

Deoxynivalenol detection and quantification for the 10 non transgenic varietal samples revealed only one positive sample. With respect to EU regulations for fungal toxins in food stuffs (EC No.1881/2006, EC No.1881/2007), the samples deem suitable for use as food and feed (*Table 1*).

Table 1 - The data for concentrations of OTA, DON, starch and proteins

Variety	DON (µg/kg)	OTA (ng/g)
Crişana	< LOD	N/A
Milcov	< LOD	< LOD
Mostiştea	143	0.06
Olt	< LOD	0.24
Rapsodia	< LOD	< LOD
F-322	< LOD	<LOD
F-125-06	< LOD	< LOD
F-254-08	< LOD	0.12
F-475	< LOD	0.03
F-225-06	< LOD	N/A

The results indicate that the concentration of deoxynivalenol in nine varieties is below the detection limit of 0.02 µg/ml. Only the hybrid Mostiştea contains identifiable levels of DON being at 143µg/kg. However, no influence of these concentrations is found on amounts of starch and proteins (*Figs. 2,3*).

Eight of the 10 selected varieties were analysed for the concentration of ochratoxin A. As the samples were obtained on the harvest day, the detected concentrations correspond to accumulation of OTA in the field conditions.

Certain mycotoxins e.g. hepatotoxins are known to disrupt the cellular functions via oxidation of key proteins (Srinivasan *et al.,* 2006). However, in this study, no influence is established for DON and OTA upon the levels of starch and proteins. The obtained data values show no significant changes in concentration of starch and protein quantities in any of the varieties as all values lie within the normal concentration range of these biomolecules in maize grains. Only the variety F-475 showed a higher value for OTA than the value permitted for infants.

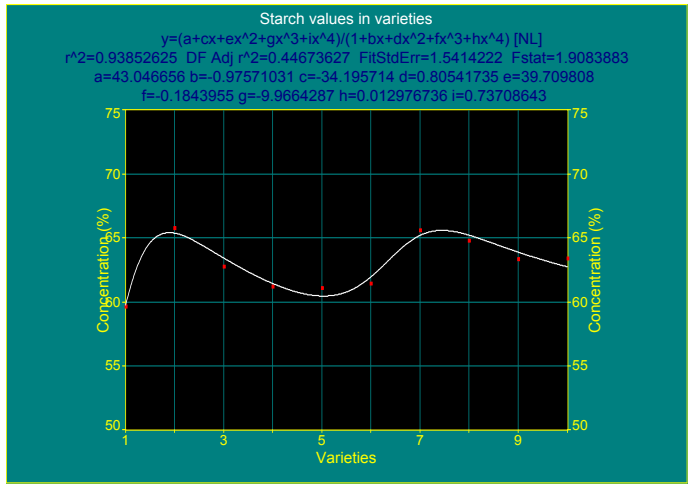

Figure 2 - The variation of concentration of starch content in varieties
Mean=62.952 std. dev.=2.072

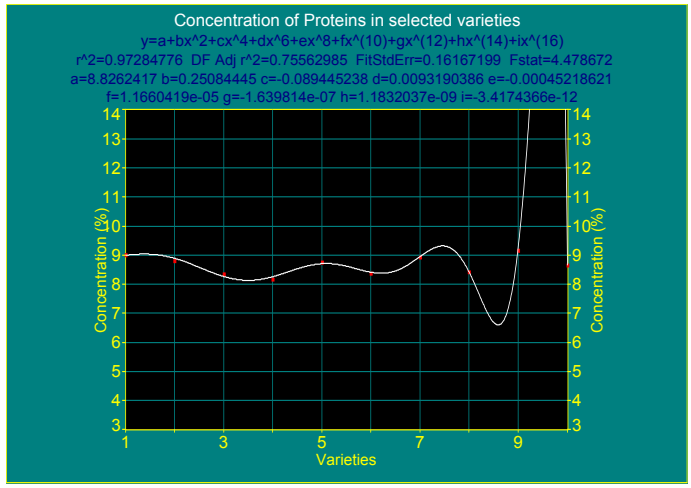

Figure 3 - The concentration of proteins in samples
Mean=8.675 std. dev.=0.326

The data is in conformation with previous results obtained in 2007, when 43 samples were collected and analysed for the presence of OTA. The results indicated that samples were positive in a significantly big proportion i.e. 24 from 30 of samples (80%). Only two positive samples had values for ochratoxin A higher than the admissible value of 5 ng/g (EC 1881/2006), the levels being at 19.92 and 11.72 ng/g. Rest of the positive samples were below than 2 ng/g (Muhammad and Angelescu, 2011).

A review of the peer studies upon the resistance potential of genetically modified maize hybrids reveal varying opinions. The results of the above experiment support the view that conventional crops are no inferior to GM counterparts with respect to permitted levels for fungal toxins. The studies by Israel-Roming *et al.* (2009) revealed that out of 125 samples of conventional maize grains (samples obtained from five wide apart counties viz: Galați, Bacău, Oltenia, Timişoara, Bihor), only 25 % samples were positive for DON and only 23% samples were positive for OTA. Moreover, the majority of the samples were found fit for human and animal consumption. Similarly in the studies by Tabuc *et al.,* (2009), conventional maize hybrids (no of samples = 54) did not contain neither fumonisins nor ochratoxin A. For an overwhelming majority of the samples, the measured values were either below the limit of detection or under permitted concentration for consumption.

CONCLUSIONS

As the interaction of fungi upon maize crop is a complex phenomenon and yet not understood well, the role

of transgenes for conferring the resistance against fungal pathogens is not yet established. If on one hand the data from the producer company has shown the reduced level of mycotoxins in Bt maize as compared with conventional hybrids, then on the other hand conventional varieties also exhibit fairly low levels for them. The concentration of mycotoxins depends upon a number of agronomic and environmental factors such as insect attacks, abundance of weeds, humidity, temperature and moisture content in grains at the time of harvest, etc. The results provide crucial evidence that the newly developed conventionally bred hybrids exhibit fairly low levels of mycotoxins in grains. The reduction in mycotoxin concentration needs to be further analysed in a variety of experiments involving ecological, agronomic and germplasm relationships for controlling or reducing the extent of fungal contamination. Such data is helpful in making decision about the biosafety status of submitted GM maize events in new regions and countries such as Pakistan where VT Double Pro GM maize event (cry1A.105+cry2Ab2) is under trials for the 3rd consecutive year.

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