Maize breeding in Croatia

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In Croatia today, maize is grown on about 400 000 ha yearly. It was first time introduced to today's Croatia during the second half of XVIth century (ROJC and KOZUMPLIK, 1996). First written document on maize presence on the Croatian territory dates back to 1777 when Taube wrote about growing maize in the regions of Slavonia and Sriem (eastern Croatia) and about its use as food for people and feed for animals. The first introduction into Croatia was most likely from Italy. First maize introductions brought to Europe as well as to Croatia were of flint type originating from the Caribbean region. Period from the first introduction to the beginning of 20th century was the period of adaptation to the Croatian pedo-climatic conditions. At the beginning of the last century the second introduction of maize to the territory of Croatia occurred. These introductions originated from the USA such as the open pollinated variety Queen of Prairie, Reid Yellow Dent, and others. This resulted in growing American dent along with the earlier introduced and adapted flint maize varieties. Natural cross-pollination between the two maize types occurred and eventually first domestic (Croatian) dent maize varieties were developed. At that time began maize breeding work in Croatia.

Breeding in the past

The first significant maize breeding success at the territory of Croatia was the open pollinated dent variety Rumski Zlatni Zuban (Ruma's Golden Dent). It was obtained from the cross between the variety Queen of Prairie and the adapted flint maize. Author of the new variety was Fleischman (about 1910), (ROJC and KOZUMPLIK, 1996).

After the World War I from the latter germplasm the varieties Beljski Zuban (Belie's Dent) and after World War II Vukovarski Zlatni Zuban (Vukovar's Yellow Dent) were developed.

In the western parts of Croatia flint rather than dent maize was asked for by the producers between the two Wars and even after the World War II. In order to grow maize in western parts of Croatia, one of the priority breeding goals of that time was shortening the vegetation period. The most popular open pollinated variety of flint type was Hrvatica. Also quite widely grown was the flint variety Osmak (Eight Row Variety) and the dent varieties Koriæev brzak (Koric's Early Dent) and Maksimirski rani zuban (Maksimir's Early Dent). Between the two World Wars two most important breeders-scientists were prof. Koric and prof. Tavcar.

After the World War II variety hybrids were grown too, i.e. Hrvatica x Osmak, Maksimir's Early Dent x Hrvatica and others.

In 1950-es North American hybrids as well as inbreds (Wf9, N6, Hy, M15, 38-11, K148, K150, Oh7, L317, A347, A375, W22, L289, Ia205, W32, W187-2, B8, Ia153, WM138, R3, W153R, W374, W37A, W182B, W79A, W75, W41A, W59E, WD, W9, W15, W85 and others) were introduced to Croatia. The American hybrids at that time lacked good adaptability to the Croatian growing conditions They were especially susceptible to *Helminthosporium turcicum*. Difficulties were experienced with seed production too. Consequently, Croatian breeders started improving latter traits of the available inbred lines and developing new hybrids. First Croatian maize breeding program was started in the Institute for Breeding and Production of Field Crops in Botinec, near Zagreb in 1947. Soon afterwards maize breeding program was started in the Agricultural Institute Osijek as well. In the Botinec Institute very early, attention was paid to resistance to leaf diseases and, artificial leaf infection was soon started. Inbreds developed from domestic germplasm had horizontal and vertical resistance to *H. turcicum*.

The first domestic inbred line Bc3, was developed from the Maksimir's Early Dent within that program and, in 1962 the first domestic hybrid Bc 590, (Wf9 x N6) x (Bc3xW153R), was released. Author of the hybrid was D. Palaveršic (PARLOV et al., 2002). The hybrid was soon accepted by growers and was grown far almost 10 years. In 1964 Osijek Institute released its first DC hybrid Os650, (Wf9xOs2)(AxW22), (RADIC et al., 1994). The breeder was Lj. Radic. In 1968 several BcSC hybrids of different FAO groups were released. Among the hybrids were BcSK5A, BcSK5AM, Bc2122, Bc4321, Bc4821 and others. First SC hybrids from the Osijek Institute were Os692, Os660, Os615, OsSK6-203, OsSK670. In addition to resistance to *H. turcicum* attention was paid to resistance to stalk rot and ear rot and to drought resistance. More and more attention was paid to resistance to stalk lodging.

During the last 50 years maize yield in Croatia has been increased for more than four times (PARLOV et al., 2002). There have been registered all together 386 domestic hybrids in the country and 40 abroad to the Croatian maize breeders (*Table 1*) (KOZUMPLIK and MARTINIC-JERCIC, 2000; PARLOV et al., 2002).

Not all the registered hybrids have been equally well accepted in the commercial production (*Graphs 1, 2*).

Present breeding work

Presently, in Croatia maize breeding is carried out in two institutes, Bc Institute for Breeding and Production of Field Crops in Zagreb which is a private firm, and in the Agricultural Institute in Osijek, a public institution. There is also a new seed establishment CT SJEME in Zagreb. In the commercial production are present also „Pioneer" hybrids with 25 to 30% and some other foreign seed companies' hybrids with a small percen-

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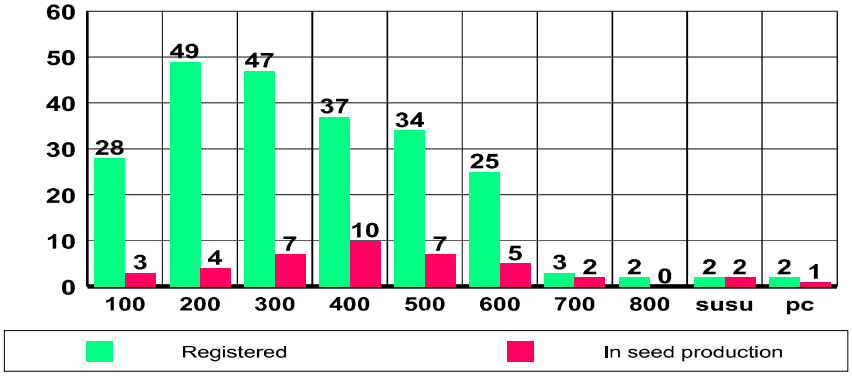
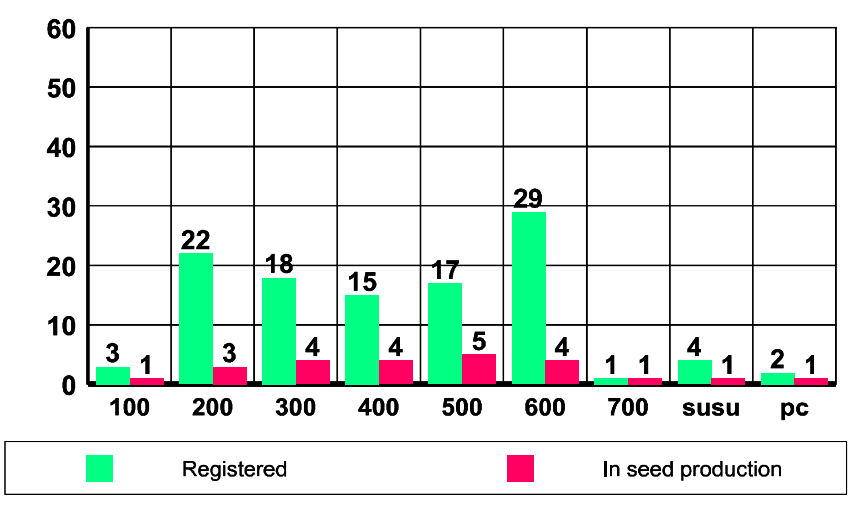
Table 1: **Number of registered domestic maize hybrids in Croatia.**

Institution	No. of registered hybrids					Total
	-1970.	1971.-80.	1981.-90.	1991.-98.	1999.-03.	
Bc Institute, Zagreb	17	62	72	66	12	229
Agricultural Institute, Osijek1340	13	40	24	13	21	111
A.F. Zagreb	3	13	4	-	-	20
PCH Zagreb	-	15	6	2	-	23
CT Seed, Zagreb	-	-	-	-	3	3
Total	33	130	106	81	36	386



Graph 1: **Bc maize hybrids registered during the 1962-2003 period and their number in seed production by FAO groups.**



Graph 2: **Os maize hybrids registered during the 1964-2003 period and their number in seed production by FAO groups.**

tage of the total production. At the Faculty of Agriculture in Zagreb research is done rather than commercial breeding. Breeding goals of Croatian breeders can be defined as „Development of better yielding inbreds and hybrids FAO 100/200 - 600/700, of good yield stability, of standard kernel quality, and hybrids for special use“ (ROJC and KOZUMPLIK, 1996). (The FAO 100 group requires about 916° C and FAO 700 about 1582° C from seeding to the physiological maturity). The main goal is improving genetic basis of inbreds and hybrids for kernel yield. Hybrids are expected to have resistance to lodging and to be suitable for mechanical harvesting.

Yield of the hybrids depends on combining ability of parental inbreds. Various germplasm has been used as source of inbred lines. For developing new inbreds breeders have used germplasm from several origins, i.e. Lancaster, BSSS, ID, domestic old varieties and synthetics. Croatian breeders are continually looking for new genetic variability. In that respect several recurrent selection programs have been carried out in the two institutes and at the Faculty of Agriculture Zagreb. Large portion of germplasm of the populations under recurrent selection is of Croatian origin.

The traits to be improved in long and short term breeding programs are mostly grain yield, fast dry down, starch content, protein content, and micronutrient content in grain, as well as resistance to lodging, resistance to biotic (*Helminthosporium turcicum*, *Colletotrichum graminicola*, *Fusarium gramienearum*, *Ostrinia spp.*, *Diabrotica spp.*) and to environmental stresses (mostly to drought).

Regarding resistance to *H. turcicum* infection is done with inoculum containing race 1 and 2 of the pathogen. *C. graminicola* is present in Croatia, more in narrow crop rotation areas. Spraying spore suspension several times in early development stages (3-5 leaves) seems to be a successful inoculum technique for most leaf diseases important in the Croatian breeding programs.

Resistance to stalk rot and lodging is a major consideration in the corn hybrid development. Fungi of the genus *Fusarium* are the most frequent pathogens of stalk rot in Croatia but, in the humid years very important pathogen is *C. graminicola*. Breeding for the diseases resistance is under the condition of natural and artificial infection. As a source of resistance to stalk rot, egzotic germplasm has been used quite extensively.

Breeding for resistance to ear rot has become very important after it was found that the micotoxins of *F. graminearum* and *F. moniliforme* were cancerous. Most of the tested inbreds are moderately susceptible.

Feeding quality of grain is also a trait that Croatian breeders have been improving. There has been found significant variability among different hybrids which

seems to be related to the breeding institution too. The differences in kernel feeding quality have been determined through animal feeding experiments.

Breeding methods

For developing new inbred lines pedigree method of selection is used mostly. Breeding populations in which selection starts are mostly F_2 populations from two inbreds. Pedigree method is also used in populations from backcrosses as well as in the populations resulting from recurrent selection. Usually in the breeding population i.e. in F_2 after crossing two inbreds about 500 plants is selfed and F3's are grown as ear per row, 20-30 plants per row. Within these, 200 plants are selfed and from the seed F_4 is grown where about 80 plants are selfed. In F_5 about 40 rows having phenotypically uniform plants are selected for selfing and for testing combining ability. Winter generations are grown in South America (Chile, Argentina). For improving qualitative traits of good inbreds, backcrossing is mostly used.

The first Croatian inbred line Bc3 was obtained from the open pollinated variety Maksimirski rani zuban (Maksimir's Early Dent). In 1947 200 plants of the variety were selfed which was the beginning of breeding the line. After six generations of selfing the line Bc3 was selected. It is of medium height, has firm thin talk, and polygenic resistance to *H. turcicum*. The inbred was one of the parental lines of the first Croatian hybrid registered in 1962. Afterwards a number of lines and numerous hybrids have been developed in Croatian maize breeding institutions (*Table 1*).

Biotechnology in maize breeding

Regarding biotechnology, it has been more the subject of research than of application in commercial breeding. Most of the research has been on using molecular markers in studying genetic variability and on the possibility of using the markers to follow the traits (alleles) under selection. Some tissue culture studies have been carried out too. Following are some of the Croatian maize research projects financed by the Ministry of Science and Technology:

- Development and improving maize populations, lines and hybrids;
- Methodical improvement of breeding process in maize;
- Recurrent selection of M3 maize synthetic - changes on phenotypic and molecular level;
- Maize heterosis expression and heritability of callus induction and somatic embryogenesis in relation to genotype;
- Breeding maize for yield and resistance to diseases;
- Integrating SSR markers in maize breeding and seed production.

Field trials

New hybrids are usually tested first in the „observation plots" test with two replications and two rows per plot, along with the control hybrids. The hybrids are observed during the vegetation period for agronomic type, earliness, lodging, etc. The plots are often harvested and kernel yield and moisture content are determined. Next year the selected hybrids are planted again in two replication trials at two to three locations, taking care that in a trial are hybrids of the same FAO group (and possibly of similar height). Next step is testing the best hybrids in a four replication trial at more locations (up to eight in Croatia). The four replication trials are carried out usually for two years. Resistance to pathogens is tested in separate nurseries.

Afterwards, the best hybrids are ready for testing in the State trials for registration. There, the hybrids are tested for three years. Breeders usually ask maize producers to test the promising hybrids at their farms too. The farmers' opinion has proven to be quite important and useful. After a hybrid is registered it can be grown commercially and care is taken of seed production. Inbred seed is produced by hand pollination to the quantity of 10 kg per line and further in space isolation. Seed production of inbred lines consists of basic seed production and production of commercial seed.

In conclusion, from the maize breeding programs in Croatia which have been mostly carried out in the Bc and Os institute, have come out hybrids which can be successfully grown in different climatic and growing conditions, for kernels and for silage. During the past years, genetic yield potential of all FAO groups has been significantly improved. A constant care is being taken of genetic variability for improving economically important traits. The breeding work is scientifically sound and contemporary. Today 41 Bc hybrids, 24 Os hybrids and one hybrid of a new Croatian maize seed firm (CT Seed Zagreb) are available to the producers for various purposes of growing. The hybrids are successfully grown in Croatia and abroad.

References

KOZUMPLIK, V., Z. MARTINIC-JERCIC, 2000: Field crops and vegetable breeding in Croatia. Agriculturae conspectus scientificus, 65(2):129-141.

PARLOV, D., I. BUHINICEK, A. VRAGOLOVIC, Z. CERGAN, 2002: Dostignuca u oplemenjivanju ranih do srednje ranih Bc hibrida kukuruza. Zbornik simpozija, Novi izazivi v poljodelstvu, pp. 135-138.

RADIC, Lj., I. BRKIC, S. VUJEVIC, 1994: Doprinos oplemenjivanja na Poljoprivrednom Institutu Osijek proizovodnji kukuruza u istocnoj Hrvatskoj od 1960. do 1993. godine., Poljoprivredne aktualnosti 30/94, (1-2):367-385.

ROJC, M., V. KOZUMPLIK, 1996: Kukuruz (Zea mays L.). Plant Breeding (book), Zagreb, pp. 207-236.

VEKIC, N., D. PARLOV, 1990: Vrijednost i perspektiva roditeljskih komponenata u hibridnim kombinacijama kukuruza. Poljoprivredne aktualnosti 35(1-2):133-142.