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Genetics and Breeding / Genetika i oplemenjivanje

Ratar. Povrt. / Field Veg. Crop Res. 47 (2010) 485-492
original research article / originalni naučni rad

Colchicine-Induced Variations in Survival Rate and Morphological Characteristics of Water Yam (*Dioscorea alata*)

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received / primljeno : 17.04.2010. revised / prerađeno : 21.05.2010. accepted / prihvaćeno: 24.05.2010.

Summary: The effects of 0.2% aqueous solution of colchicine on the survival rate and morphological features of five accessions of *Dioscorea alata* were investigated. Sprouting buds of two month old plants were treated with 0.2% colchicine and their performances were monitored until maturity. Survival of buds was lower in all the treated plants (ranging between 6.25% and 8.75%) compared to controls (between 13.25% and 15.25%). However, colchicine treated vines had higher survival rates when exposed to drought (13.8% - 24%) compared to controls (7.2% - 16.69%). Higher number of leaves, larger leaf width and fewer numbers of stomata were observed among the treated plants. The survival of buds between the treated plants and control plants was significantly different at $P \leq 0.05$. The treated and non-treated plants were also significantly different at $P \leq 0.05$ for leaf width in accessions TDa02/00246, TDa98/00116 and TDa99/00240, and for stomata number in accessions TDa02/00151 and TDa02/00246. Our results suggest that colchicine can be used to induce mutagenic changes in yam which may be of agronomic importance in the production of the crop.
Key words: colchicine, *Dioscorea alata,* drought tolerance, morphological variations, polyploidy, tubers

Introduction

Water yam (*Dioscorea alata* L.) is an important tuber crop and is a staple food for millions of people in tropical and sub-tropical countries (Hahn 1995). It was first cultivated in South-East Asia but is now grown in Africa, the Pacific Island, and the West Indies. Although it is not grown to the same extent as the Africa yams (*D. rotundata* and *D. cayensis*), it has the widest global distribution than any other cultivated yams (Mignouna 2003). In the United States it has become an invasive species in some southern states. In the Philippines it is known as "Ube" (or Ubi) and is used as an ingredient in many desserts. In India it is known as "ratalu" or violet yam or the "moraga surprise", while in Hawaii it is known as "Uhi".

It was brought to Hawaii by the early Polynesian settlers and became a major crop in the 1800s when the tubers were sold to visiting ships as an easily stored food supply for their voyage (White 2003).

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The plant has a long history of asexual propagation. Dioecy, irregular and erratic flowering, asynchrony of sexual seed development and apparent paucity in seed set has led to the species being regarded as sterile (Coursey 1967). However, with the development of artificial pollination under optimized cultural management practices (IITA 1996) and a better understanding of yam flowering biology, large volumes of viable *D. alata* seeds can now be produced. This has opened up the opportunity for conventional plant breeding in *D. alata.* The conventional breeding method which has been applied in *D. alata* is the hybridization method, which may take a very long period for scientists to achieve homozygous genotype. Unfortunately, not much improvement in *D. alata* has been achieved through conventional breeding methods. They are laborious and time consuming because yam anthers are so small and difficult to pollinate, and also to achieve homozygous genotype will take a very long period of time due to cross pollination. Therefore the use of chemical agents in inducing mutation which can lead to chromosome doubling or polyploidization with possible increase in yield, quality and adaptation (mutation breeding) is desirable (Dewey 1979, Castro et al. 2003, Burun & Emiroglu 2007).

With the discovery of "colchicine technique" and oryzalin, for inducing polyploidy, breeders have seized upon this unconventional technique as means of plant improvement (Hancock 1997). Colchicine can be used to restore fertility in interspecific hybrids or induce polyploidy as genetic bridge for crossing between species of different ploidy levels (Mehetre et al. 2003). The colchicine technique is attractive for the following reasons: (1) it may enable the isolation of homozygous or near-homozygous genotypes from hybrids more rapidly than the conventional breeding, (2) it may generate new genetic variation by chromosome loss, rearrangement or gene mutation, thereby enlarging the breeder's germplasm base, and (3) it is a simple, inexpensive technique requiring no special equipment or expertise (Luckett 1989).

Colchicine mutagenesis at inducing polyploidy has successfully been exploited in various plant species, such as barley (Gilbert & Patterson 1965), azalea (Pryor & Frazier 1968), phaleanopsis (Griesbach 1981), iris (Yabuya 1985), cotton (Luckett 1989), cranberry (Dermen & Henry 1994), cyclamen (Ishizaka & Uematsu 1994, Takamura & Miyajma 1996) and *Brachiaria brizantha* (Pinheiro et al. 2000), *Sorghum bicolor* (Ghaffari 2006), *Cicer arietinum* (Pundir 1983), *Acacia* species (Blakesley et al. 2002), *Colccinia palmate* and *Lagenaria sphaerica* (Nituli & Zobolo 2008), *Nicotiana tabacum* (Burun et al. 2007), *Chamelaucium uncinatum* (Yan 2001). It has also been used to overcome interspecific incompatibilities in some *Gossipium species* (Mehetre et al. 2003, Rauf et al. 2006), to restore fertility in interspecific hybrids of *Manihot* species (Nassar 2002), *Xanthosoma* species (Tambong et al. 1997) and *Viola* x *Wittrockiana* (Ajalin et al. 2002).

Timing and concentration is also important when colchicine is being used to induce polyploidy in plant, since not all plants respond to colchicine in the same way. Colchicine has been used in different concentrations and timing to induce polyploidy. In chickpea, it was most effective at inducing autotetraploidy at 0.25% concentration at 4 h time duration (Pundir et al. 1982). The quantity of 50 mg l^{-1} of colchicine was effective at inducing polyploidy in *Phalaenopsis* (Griesbach 1981). Colchicine dropping treatment at a concentration of 2.000 mg l^{-1} for one day led to the highest rate (14%) of tetraploid formation in the interspecific hybrid between *Dianthus caryophllus* L. and *D. japonicus* Thumb. (Nimura et al. 2006). Treating petiole sheaths with 1.25 mM colchicine induces solid polyploids and cytochimeras in cocoyam tissues (Tambong et al. 1997). Tumor-like growths were produced at the nodes of cuttings of *Tradescantia poludosa* by complete immersion for 48 h in aqueous solution of colchicine (Mc Gowan & Bishop 1953). Fertility of sterile interspecific hybrids between cassava and wild *Manihot* species was restored by chromosome duplication following application of colchicine at 0.2% aquaeous solution for a period of 24 h and reached 95% of viable pollen in tetraploid types compared to 13% in the diploid forms (Nassar 2002).

An enlargement of vegetative organs is a common feature of induced polyploids (Allard 1971). This phenotypic effect has been described in ryegrass (Myers 1939), sorghum (Franzke & Rose 1952), flax (Dirks et al. 1956), cotton (Luckett 1989, Rauf 2006), cassava (Nassar 2002). Studies comparing untreated and colchicine treated ryegrass genotypes, without the occurrence of chromosomal duplication, have shown significant differences in many traits, e.g. leaf area, flowering date and flower number (Hague & Jones 1987), leaf weight (Francis & Jones 1989); mesophyll cell size and chloroplast number (Francis et al. 1990, Hassan et al. 1991). These investigations suggest a mutagenic action independent of changes at ploidy levels.

The objectives of this research were to determine the influence of colchicine on: (1) survival of buds of *Dioscorea alata* genotypes, (2) morphological characters of *Dioscorea alata* genotypes and (3) survival rate of *Dioscorea alata* vines exposed to drought.

Materials and Methods

Tuber collection. Tubers of five accessions of *Dioscorea alata*, (TDa02/00151, TDa02/00246, TDa98/01176, TDa98/01166 and Tda99/00240) were collected from the yam breeding unit at IITA and were cut into mini sets of about 100 g per set. The mini sets were air dried for 24 hours before planting to prevent decaying from the surface.

Planting of sets. The air dried tuber sets were planted in the polyethylene bags of about 2000 cm^3, filled with sandy-loam soil in the screen house. The sets were planted according to the arrangement of the polyethylene bags. One set was planted per bag per treatment per accession. Each accession had six treatments, (control, 0.1%, 0.15% 0.2%, 0.25% and 0.3%) of colchicine concentration. Each treatment contained ten plants and was replicated four times. The experimental design was complete randomized design.

Colchicine treatments. When the plants were exactly two months old, they were trimmed to two nodes each. Colchicine was dissolved in sterile water in a ventilated weighing station to the

above mentioned concentrations. Sprouting buds of each plant were plugged with cotton wool. The cotton plugs were held in place with parafilm. Each plugged bud was soaked with appropriate colchicine solution twice a day for 48 hours by the use of a micro-syringe. The control plants were soaked with distilled water. Each sprouting bud was treated four times.

Two months after colchicine treatments, data were collected from the vines originated from the treated buds on the survival of buds, number of leaves, leaf length and leaf width.

Vine propagation. In the third month after colchicine treatment, the vines which grew from the treated buds were made into cuttings of two nodes each and transferred to the field for propagation where they were exposed to drought (dry season). Two vines were propagated per plant per colchicine treatment per accession, six colchicine treatments were involved per accession and the total number of accessions was five. The total number of vines transferred to the field was 2400. The experiment was laid out in a complete randomized design with four replications (Fig. 1 and 2). Two

Figure 1. Field plot where the vines exposed to drought were grown
Slika 1. Parcela sa gajenim reznicama izloženim suši

Figure 2. Exposure of vine cuttings derived from colchicine treated sprouted buds to drought
Slika 2. Izlaganje reznice dobijene tretiranjem pupoljaka kolhicinom sušnim uslovima

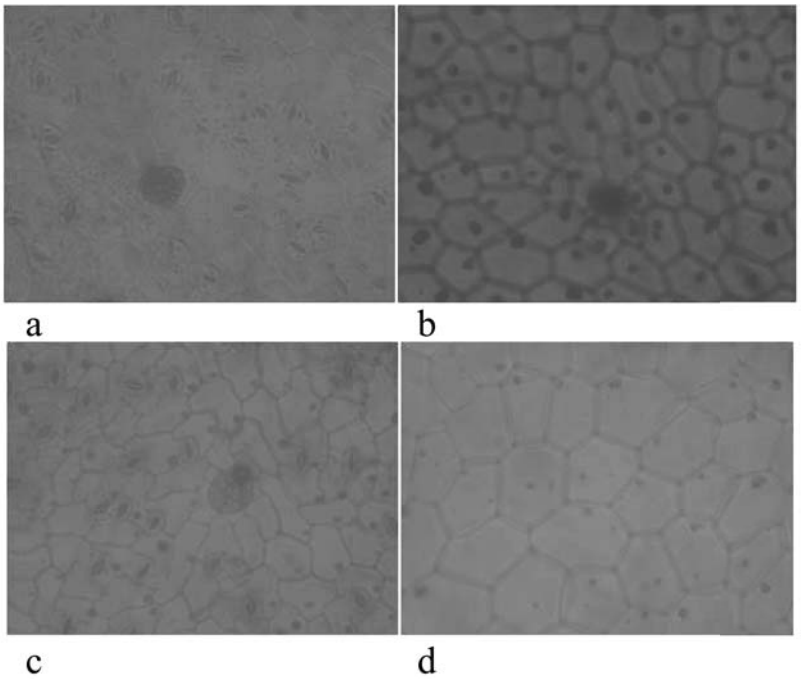

Figure 3. Epidermal cells of control (a and b) and colchicine-treated (c and d) *Dioscorea alata* obtained from abaxial (a and c) and adaxial (b and d) leaf surfaces
Slika 3. Ćelije epidermisa kontrolne (a i b) i tretirane (c i d) varijante *Dioscorea alata* sa abaksijalne (a i c) i adaksijalne (b i d) površine lista

hundred cm^3 of water was applied to each plant of both treated and control twice a day.

Six months after vine propagation, data were taken on the percentage survival of vines and the stomata number and stomata index were taken from the leaves of the surviving plants.

Because of the high death rate of plants on the field, data could only be compared between the control and the 0.2% colchicine treatment for all the accessions, since they were the only remaining survivors on the field.

Stomata assessment. To obtain epidermal peels, two matured leaves were obtained from the top of both treated and controls of established plants. Rectangular cuttings were made from the two leaves by the use of scissors. The leaf cuttings were placed in petri-dishes and nitric acid was poured on the leaf cuttings to separate the adaxial part from the abaxial part. The petri-dishes were covered and placed in the sun for about 30 minutes.

The transparent epidermal peels were later properly rinsed in water. The strips were stained in safranin, excess stain was rinsed off with water for easy viewing. Painting brush was used to guide the strips onto a clear glass slide in water. The glass slides were gently removed from water and a drop of glycerine was placed on each epidermal strip on each slide. Cover slip was placed over each slide and the prepared slides were examined under a microscope at 100x magnification. The number of epidermal cells, stomata and stomata indices on two leaves of each treatment and in 40 randomly chosen microscopic fields (area 1 mm^2) of each leaf peeling was recorded. Therefore, each figure in the text is a mean based on 80 microscopic fields.

Statistical analysis. The results were analyzed using ANOVA and least significant difference (LSD), $P \leq 0.05$ level of significance was used to compare the differences in means between the treatments.

Results and Discussion

Results were based on the comparison between only the control and 0.2% colchicine treatment, since they were the only surviving treatments on the field.

Colchicine treatment reduced the survival of buds in all the accessions, which resulted in the failure of some plants to induce shoots at the treated nodes. In all five accessions, survival rates of the treated buds were lower in comparison to the control (Tab. 1).

Number of leaves. The treated plants of *D. alata* had more leaves than the control in most of the

Table 1. Number of survived buds (mean ± standard error and coefficient of variation) of the control and colchicine-treated *Dioscorea alata*
Tabela 1. Broj preživelih pupoljaka (srednja vrednost ± standardna greška i koeficijent varijacije) kontrole i kolhicinom tretirane *Dioscorea alata*

Accession Genotip	TDa 02/00151	TDa02/00246	TDa98/01176	TDa98/01166	TDa99/00240
Control Kontrola	13.25±2.25a 22.20	14.75±1.93a 26.20	15.00±1.08a 14.00	15.25±1.50a 16.00	13.75±1.65a 24.00
Treated Tretirane	8.75 ±1.49b 34.37	7.50 ±1.85b 49.00	6.25 ±1.18b 37.00	8.25 ±0.75b 18.00	8.50 ±1.30b 29.00

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Table 2. Number of leaves (mean ± standard error and coefficient of variation) of the control and colchicine-treated *Dioscorea alata*
Tabela 2. Broj listova (srednja vrednost ± standardna greška i koeficijent varijacije) kontrole i kolhicinom tretirane *Dioscorea alata*

Accession Genotip	TDa 02/00151	TDa02/00246	TDa98/01176	TDa98/01166	TDa99/00240
Control Kontrola	31.70 ± 2.74a 17.37	27.25 ±1.14a 8.30	22.90±2.50a 21.83	22.35±1.56a 14.00	14.65±0.46a 6.27
Treated Tretirane	34.42 ± 2.70a 15.69	32.85±2.20a 13.36	20.81±1.57a 15.00	25.36±1.90a 13.60	17.43±1.84a 21.20

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Table 3. Leaf length (mean ± standard error and coefficient of variation) of the control and colchicine-treated *Dioscorea alata*
Tabela 3. Dužina listova (srednja vrednost ± standardna greška i koeficijent varijacije) kontrole i kolhicinom tretirane *Dioscorea alata*

Accession Genotip	TDa 02/00151	TDa02/00246	TDa98/01176	TDa98/01166	TDa99/00240
Control Kontrola	7.13 ± 1.01a 28.20	6.77 ± 0.62b 18.30	6.76 ± 0.42a 12.60	7.06 ± 0.68a 17.90	6.05 ± 0.73a 24.00
Treated Tretirane	5.55 ± 1.21b 43.80	8.49 ± 1.10a 26.00	6.36 ± 0.16a 5.00	7.35 ± 0.80a 21.80	4.95 ± 1.17b 47.00
Difference Razlika	-12%	11%	-3%	2%	-10%

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accessions except for TDa98/01176 where leaf number was fewer in the treated plants. There was a significant ($P \leq 0.05$) treatment effect for number of leaves but the difference in mean number of leaves between control and the treated plants was not significant for all the accessions (Tab. 2).

Leaf length and width. Accessions TDa02/00246 and TDa98/01166 increased in leaf length by 11% and 2% respectively, following colchicine treatment compared to their controls while others had reduced length following colchicine treatment. The difference in means of the treated and the control of TDa02/00246, TDa02/00151 and TDa99/00240 were all significant ($P \leq 0.05$) while the difference in means between the control and the treated plants of accessions TDa98/01166 and TDa99/01176 were not significant. Moreover, all the treated *D. alata* had larger leaf width ranging from 0.09% to 13% increase in width. There was significant ($P \leq 0.05$) colchicine effect on the leaf width for TDa02/00246, TDa98/01166 and TDa99/00240 but not for TDa02/00151 and TDa98/01176 (Tab. 3 and 4).

Survival rate of vines on the field. The survival rate of vines of *D. alata* exposed to the drought condition in the field was low in all the accessions. Within an accession, survival rate was higher in the treated plants compared to the control across all the

Table 4. Leaf width (mean ± standard error and coefficient of variation) of the control and colchicine-treated *Dioscorea alata*
Tabela 4. Širina lista (srednja vrednost ± standardna greška i koeficijent varijacije) kontrole i kolhicinom tretirane *Dioscorea alata*

Accession Genotip	TDa 02/00151	TDa02/00246	TDa98/01176	TDa98/01166	TDa99/00240
Control Kontrola	5.30 ±0.55a 20.80	4.91±0.45b 18.00	5.34±0.62a 23.60	5.55±0.46b 16.40	4.41±0.49b 22.00
Treated Tretirane	5.46 ±0.69a 25.40	6.37±0.69a 21.70	5.35±0.75a 28.00	6.37±0.49a 15.50	5.08±0.39a 15.00
Difference Razlika	1.5%	13%	0.09%	6.9%	7%

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Table 5. Percentage of survival of vines of the control and colchicine-treated *Dioscorea alata*
Tabela 5. Postotak preživelih reznica kontrole i kolhicinom tretirane *Dioscorea alata*

Accession Genotip	TDa 02/00151	TDa02/00246	TDa98/01176	TDa98/01166	TDa99/00240
Control Kontrola	7.2	16.69	14.3	0	10.3
Treated Tretirane	13.8	24	16.7	0	13.8

Table 6. Number of stomata (mean ± standard error and coefficient of variation) of the control and colchicine-treated *Dioscorea alata*
Tabela 6. Broj stoma (srednja vrednost ± standardna greška i koeficijent varijacije) kontrole i kolhicinom tretirane *Dioscorea alata*

Accession Genotip	TDa 02/00151	TDa02/00246	TDa98/01176	TDa98/01166	TDa99/00240
Control Kontrola	36.25 ±1.19a 19.9	36.77±0.96a 11.20	30.55±0.97a 9.96	—	29.71±1.18a 10.8
Treated Tretirane	30.27 ±1.34b 10.37	22.79±1.17b 8.90	29.97±1.11a 13.90	—	28.13±0.55a 12.6
Difference Razlika	-8.90%	-23.5%	-0.96%	—	-2.7%

Figures followed by similar alphabet in a column are not significantly different from each other
Brojevi praćeni istim slovom u koloni nisu međusobno značajno različiti

genotypes. Genotype TDa98/01166 for treated and control did not survive in the field (Tab. 5).

Stomata evaluation. Colchicine treatment caused variations in the number of stomata and epidermal cells on the different leaf surfaces. The abaxial surfaces of all the treated plants contain fewer stomata and epidermal cells in comparison to the control (Fig. 3a and 3c). There were no stomata on the adaxial surfaces of all the accessions in both treated and control plants (Fig. 3b and 3d). However, the shapes of the adaxial epidermal cell range from pentagonal to hexagonal. The adaxial epidermal cells of the treated were fewer to the control when compared. The abaxial surfaces of all the accessions both treated and control had irregular shaped cells. The mean number of stomata per 1 mm^2 area of leaf and stomata of both treated and control plants of all the accessions are shown in table 6. There were significant ($P \leq 0.05$) differences in the number of stomata between the treated and the control of TDa02/00151 and Tda02/00246, but stomata number was similar in other accessions.

Plant growth inhibition and occasional mortality is a known phenomenon in plants after colchicine treatment (Yan 2001, Rauf et al. 2006). This supports that colchicine can cause death of plants due to its toxic effect (Navarro-Alvarez et al. 1994). The suppression of growth observed among the treated plants at the first month of treatment is common after colchicine treatment

is caused by the reduced rate of cell division, in plants and it has been reported by many researchers (Blakeslee & Avery 1937, Obute et al. 2007). Successful colchicine treatment results in plants that have gigantic characteristics such as thicker-wider leaves with bigger and fewer stomata number (Uhlik 1981). It was found that the treated plants had wider leaves across the genotypes and also that the stomata number per millimeter leaf area is also related to the leaf width, in that it decreased as the leaf width increased across the genotypes and this is also related to the findings of Yan (2001) who discovered that as the leaf thickness increased in treated wax flower, the stomata density decreased. Fewer stomata number observed in the treated abaxial surfaces of all the treated *D. alata* were evidence of successful colchicine treatment, and these results are in agreement with the works of Schuiz-Schaeffer (1985), Allard (1960), Stebbins (1950), Davis & Heywood (1967), Ugborogho & Sodipo (1985), Ugborogho & Obute (1994) and Ndukwu & Obute (2006). Micro-morphological features are rarely affected by environmental factors, thus the changes in these features are due to alteration in chromosome numbers in the treated plants (Ugborogho 1982). Colchicine mutagenesis has been used in horticulture as a breeding tool to enhance ornamental characteristics such as plant size, leaf thickness and increased width to length ratio of leaves (Shao et al. 2003). The increased number of leaves among the treated plants indicates that colchicine can be used to induce bushy habit in plants and this is in support of works by Blakeslee & Avery (1937), Hewawasam et al. (2004) and Obute (2006).

The higher survival percentage of vines experienced across the genotypes among the treated plants exposed to dry weather showed that colchicine can induce drought resistance in crops. This is in support with the work of Ntuli & Zobodo (2008) on the effect of water stress on the growth of colchicine induced polyploidy *Colccinia palmata* and *Lagenaria sphaerica* and concluded that colchicine technique can be used to make drought resistant plants available to farmers.

Conclusions

Although there were changes in morphological characters induced by colchicine in all the genoypes of *D. alata*, nevertheless, further studies must be carried out to elucidate the effects of colchicine on tuber yield, nuclear DNA contents and ploidy level of *Dioscorea* populations.

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Promene stope preživljavanja i morfoloških odlika vodenog jama (*Dioscorea alata*) izazvane kolhicinom

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Izvod: Ispitivani su mutageni uticaji 0,2% vodenog rastvora kolhicina na preživljavanje i morfološke osobine pet genotipova jama (*Dioscorea alata*). Pupoljci biljaka starih dva meseca tretirani su sa 0,2% kolhicinom, a promene su praćene do zrelosti. Stopa preživljavanja tretiranih biljaka (6,25%-8,75%) bila je niža u odnosu na kontrolu (13,25-15,25%). Tretirane biljke imale su višu stopu preživljavanja kada su bile izložene suši (13,8%-24%) u odnosu na kontrolu (7,2%-16,69%). Veći broj listova, veća širina lista i manji broj stoma uočen je kod tretiranih biljaka. Vrednosti stope preživljavanja pupoljaka između tretiranih biljaka i biljaka kontrole značajno su se razlikovale pri $P \leq 0,05$. Tretirane i netretirane biljke razlikovale su se pri $P \leq 0,05$ i za širinu lista, kod genotipova TDa02/00246, TDa98/00116 i TDa99/00240, i za broj stoma, kod genotipova TDa02/00151 i TDa02/00246. Naši rezultati pokazuju da kolhicin može da se koristi za izazivanje mutagenih promena kod jama koje mogu da budu od agronomskog značaja za ovaj usev i njegovu proizvodnju.

Ključne reči: *Dioscorea alata,* kolhicin, krtole, morfološke promene, otpornost na sušu, poliploidija