hypothetical seed orchard that is consisting of 20 clones, 20 ramets per clone, and a total of 400 trees. If we bulked needle samples of four trees as done for this study, the workload varies depending on the level of mislabeling as follows. If 2% of trees are mislabeled, the suspicious bulked samples are maximum 8. Therefore we must analyze 100 bulked samples and 32 individual trees (4 trees x 8 suspicious bulked samples) and the workload is 33% comparing with individual RAPD analyses of 400 trees. If 5% and 10% of trees are mislabeled, the workload is similarly calculated as 45% and 65%, respectively. If 20% of trees are mislabeled, the suspicious bulked samples are maximum 80. In this case, we must analyze 100 bulked samples and 320 individuals (4 individuals x 80 suspicious bulked samples). In this case, the bulking procedure is not as efficient as individual analyses. However, the level of mislabeling in most previous reports is less than 10% (ADAMS, 1983; HARJU and MUONA, 1989; WHEELER and JECH, 1992), therefore we conclude a bulking procedure will be useful for checking the genetic identity of ramets in most clonal seed orchards.

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New Genotypes Development of *Populus euphratica* OLIV. Using Gametoclonal Variation

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Abstract

In order to induce genetic variation of *Populus euphratica* OLIV. through gametoclonal variation, the anthers containing microspore in tetrad-uninucleate stage were isolated and cultured in MS (MURASHIGE and SKOOG, 1962) media. Five treatments of growth regulators and two incubation conditions (light and dark) were used to induce callus from microspores. Significant differences were observed between light and dark incubation for callus induction at 0.01 level. Higher percentage of callus induction was observed in dark incubation (90%). MS medium supplemented with 2 mg/l 2,4-D and 0.5 mg/l kinetin produced most calli from microspores. The calli were subcultured for 8 months in MS medium to increase variation among regenerated plantlets. Highest plant regeneration was observed in MS medium containing 2 mg/l BA and 0.1 mg/l NAA. Five hundred and thirty-six *in vitro* plantlets were regenerated from long-term callus culture. At least 42 of the regenerants differed in some way from the original clones (7.8%). High degree of morphological variation (leaves and stems) was observed among regenerated plants. Cytological analysis on root tips of regenerated plantlets showed the presence of haploid, diploid, aneuploid and polyploid plantlets.

Key words: Populus euphratica, gametoclonal, tetrad- uninucleate, aneuploid, polyploid and haploid.

Introduction

Before 1980, plant breeders could increase the genetic variability of the basic material available to them in two ways: by crossing or by mutation. Research conducted in the 1970's proved that cell cultures derived from somatic tissues could serve as a new variability-increasing source termed somaclonal variation (HESZKY et al., 1992). The variation has also been observed among gametic-derived plants termed gametoclonal variation (MORRISON and EVANS, 1987). There are different approaches to create *in vitro* variation: (1) Long-term callus culture (BRAR and JAIN, 1994; SUMIYAET et al., 1988); (2) Using of 2,4-D as a mutagens to increase the frequency of somaclonal variation (DOLEZEL, 1984); (3) *in vitro* colchicine treatment to induce chromosome doubling (BARNABAS et al., 1991; BOUSSOUTROT and HANSEN, 1985); (4) screening for desirable somaclones for tolerance to various biotic and abiotic stresses using toxic levels of pathotoxines, herbicides, salts, etc. (BINH et al., 1993; HANSEN et al., 1994; WINICOV, 1991; BRAR and JAIN, 1988).

The most important sources of variability are the different molecular changes (gene amplification, DNA-methylation, transposable elements, etc.), chromosomal transposition changes (breakage, reunion, translocation, rearrangement, etc.) and karyotypic changes (polyploidy, aneuploidy) (HESZKY et al., 1992). Some of this variation has been shown to be heritable and to occur at a high frequency and after only a short time in culture. Chromosomal doubling, aneuploidy, chromosome structural changes and other qualitative and quantitative genetic changes were observed in alfalfa cells or protoplasts culture (BINGHAM and MCCOY, 1986). Several useful somaclonal variants have been released as cultivars in rice, wheat, red clover, maize and sugarcane (BRAR and JAIN, 1988; SKIRVIN et. al., 1994). The present study aimed to induce morphological variants in *P. euphratica* forest trees by long term callus culture derived from microspores.

Materials and Methods

Plant source

Flower buds of *Populus euphratica* OLIV., with chromosome number 2 n = 38, were collected from field-grown plants on 15 March, 1999 and stored at 4 °C for 6 d. The buds were then surface sterilized in 70% (v/v) ethanol for 1 min, followed by sodium hypochlorite solution (0.5% active chlorine in water) for 15 min, and finally rinsed three times in sterile distilled water. The stage of pollen development was checked in one anther per catkin by acetocarmine method (JAFARI et al., 1995). Buds with microspores at the unicellular stage were selected, aseptically removed and then placed in culture.

Conditions for callus induction

The medium for callus induction was MS (MURASHIGE and SKOOG, 1962) supplemented with 1,2 and 3 mg/l 2,4-dichlorophenoxy-acetic acid (2,4-D) plus 0.1 and 0.5 mg/l kinetin. The pH was adjusted to 5.8 with 1 N HCL or 1 N NaOH before autoclaving (20 min. 120 °C). All culture media were supplemented with 3% sucrose and 0.6% agar. The medium was then dispensed in sterile petri dishes (100 mm x 15mm).

The callus induction experiments were designed as a randomized complete block with two incubation conditions (light and dark) in the growth room, five hormonal treatments and four replications. Twenty-five anthers were placed singly in petri dishes containing 10 ml of callus induction medium as experimental units. Cultures were incubated in a photoperiod of 16-h light, 8-h dark, for 42 days at 25–26 °C, with light intensity of 4500–5000 lux (cool white). The percentage of anthers producing calli in each petri dish after four weeks of culture was recorded. Means separation was performed by DUNCAN's multiple range tests. Long – term callus cultures (8 months old) were then conducted in the MS containing 2 mg/l 2,4-D and 0.5 mg/l kinetin and kept in 16-h photoperiod. The callus was subcultured in the same incubator every 4 weeks.

Plant recovery and hardening

Eight months old green calli were used for shoot formation. The MS medium supplemented with 0.1,1,2,2.5 and 3 mg/l BAP (6-benzylaminopurine) plus 0.1 and 0.5 mg/l NAA (alpha-naphthaleneacetic acid) was used for shoot induction. Five callus clumps were cultured in each of petri-dishes as experimental unit. The shoot induction experiment was designed as a randomized complete block with eight hormonal treatments and four replications. The number of calli, which induced shoots, was recorded once a week for 7 weeks. The shoots derived from callus were rooted in half-MS agar medium supplemented with 0.1 mg/l indole-3-butyric acid (IBA) and 0.1 mg/l NAA. The plantlets were subsequently transferred to the greenhouse in pots containing peat, sand and soil in the ratio of 1:1:1 for the hardening phase.

Ploidy checks

Ploidy checks on the regenerated plants were root-tip cell chromosome counts. For root-tip cell squashes, harvested root-tips were immersed for 3 h in 0.002 M 8- hydroxyquinoline and colchicine 0.1 % solution. They were fixed in 3:1 absolute ethanol and glacial acetic acid at 4 °C for a minimum of 12 h. They were then macerated in 1 N HCl at 60°C for 6–10 min and rinsed in distilled water before squashing and staining with acetoorcein and acetocarmin. There up to six cells of one to three root- tips per plants were used for ploidy checks.

Results and Discussion

After 10 days in culture, anthers swelled and microspores emerged. Microspores subjected to the surface of the medium, started to produce callus (*Fig. 1 A-B*). Significant differences were observed between darkness and light photoperiod at 0.01 level (*Table 1*). The amount of callus produced in light photoperiod was less than that obtained in darkness. Effect of growth regulators on callus induction success depends very often on combinations and concentrations. Results in *Table 1* demonstrated that there were significant differences between growth regulator concentration for callus induction at 0.01 level. Mean effect of growth regulators on callus initiation from cultured anthers indicated that 2 mg/l 2,4-D with 0.5 mg/l kinetin produced high percentage of callus induction and showed significant differences to the other hormonal treatments (*Table 2*).

Table 1. – Analysis of variance for the percentage of anthers which produced calli.

Source of variation	Df	SS	MS	F
Rep.	3	134.675	44.892	0.7822
Media.	4	6143.900	1535.975	26.7630 **
(L&D)	1	9517.225	9517.225	165.829 **
Med.x (L&D)	4	226.400	56.600	0.9862
Error	27	1549.575	57.39	
Total	39	17571.775		

** = Significant F-value at the 0.01 level
L = light (photoperiod of 16-h light and 8-h dark)
D = dark

Increasing of 2,4-D from 2 mg/l to 3 mg/l did not effect on percentage of callus initiation but reducing kinetin concentration from 0.5 mg/l to 0.1 mg/l with the same amount of 2,4-D showed great effect on callus induction (*Table 2*). The combina-

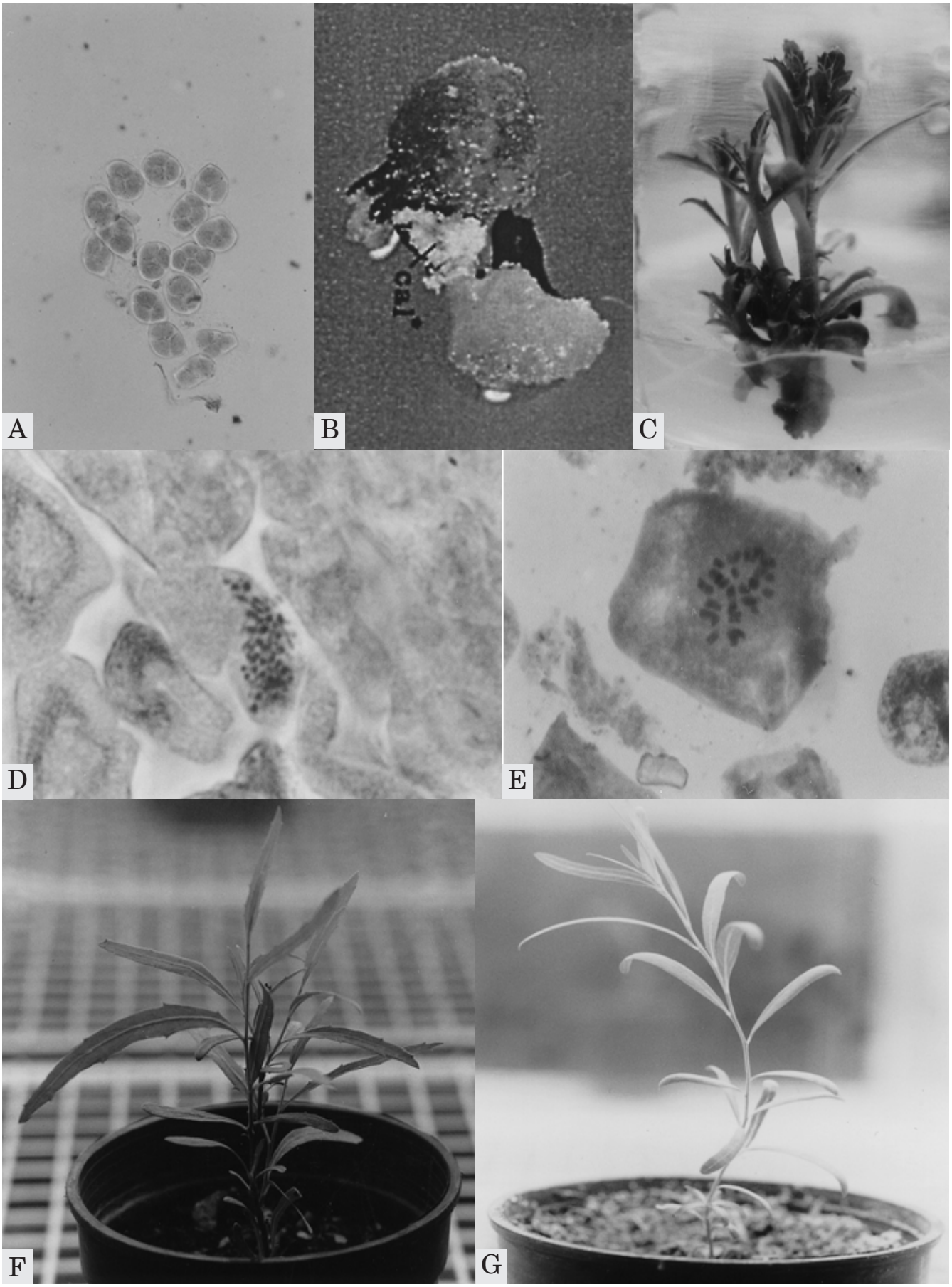
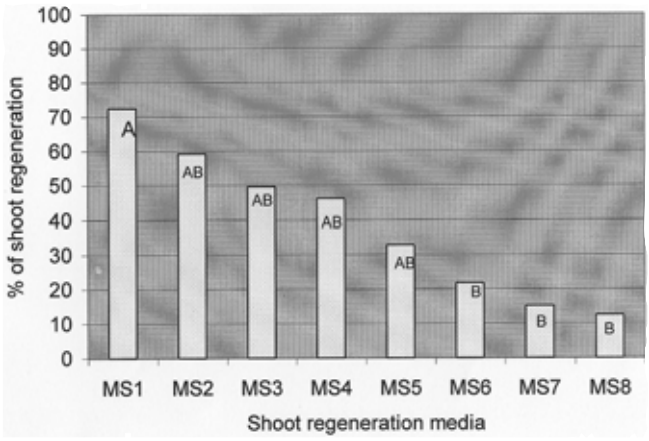


Figure 1. – Development sequences of isolated anthers to plantlets, (A) Microspores at tetrad stage, (B) Callus initiation from microspores, (C) Plantlets regeneration from long-term callues culture, (D, F) Plantlets with polyploid chromosome number (56), (E, G) Plantlets with aneuploid chromosome number (23).

tion of 2,4-D and kinetin was used to induce callus in poplar by HO et al. (1983); STOEHR and ZSUFFA (1990); JAFARI et al. (1995). MS medium containing 2 mg/l 2,4-D and 0.5 mg/l kinetin was then used for eight months of callus culture (eight subcultures). Similar long period callus culture to induce genetic variation in poplar (*Populus nigra*) was used by SUMIYAET et al. (1988). Shoot regeneration from callus culture was observed within 45 days in shoot regeneration media (*Fig. 1C*). The calli were very compact, nodular and became greenish in color after two weeks in regeneration media. Highly significant differences were observed between callus induction media at 0.01 level (*Table* 3). DUNCAN's multiple range test on mean effect of media and growth regulator treatments on shoot formation from long-term callus culture indicated that MS medium containing 2 mg/l BA plus 0.1 mg/l NAA shows significant differences to some of the other shoot regeneration media and produced highest plantlet regeneration (*Fig. 2*).



Means followed by different letters are significantly different at p = 0.01 level using Duncan's multiple range test

% Of shoot regeneration was calculated on total number of callus producing plantlets
÷ total number of cultured callus clumps multiplying by 100

MS1=BA 2 mg/l +NAA 0.1 mg/l
MS2=BA 2.5 mg/l
MS3=BA 3 mg/l
MS4=BA 2mg/l +NAA 0.5mg/l
MS5=BA 1 mg/l +NAA 0.2 mg/l
MS6=BA 0.1 mg/l +NAA 0.1 mg/l
MS7=BA 2 mg/l +NAA 0.1 mg/l + 2,4-D 0.5 mg/l
MS8=BA 1 mg/l +NAA 0.1 mg/l

Figure 2. – Mean effect of shoot regenation media on percentage of callus producing shoots.

Table 2. – Mean effects of growth regulators, dark and light on callus initiation of microspore culture in *Populus euphratica* OLIV.

MS + 2.4-D + Kinetin	% of anthers produced callus		Mean (% anthers which produced callus)
	Dark	Light	
MS +2 mg/l +0.5 mg/l	95	70	82.50 A
MS +1 mg/l +0.5 mg/l	96	68	82.00 A
MS +3 mg/l +0.1 mg/l	98	60	79.38 A
MS +3 mg/l +0.5 mg/l	95	62	78.50 A
MS +2 mg/l +0.1 mg/l	65	35	50 B

Means followed by different letters are significantly at $\alpha = 0.01$, following DUNCAN's multiple range test.

Table 3. – Analysis of variance for plant regeneration from long-term callus culture.

Source of variation	Df	S.S	M.S	F
Replication	3	1106/028	368/676	2/838
Treatment	8	13223/034	188/005	14/5428 **
Error	21	2727/754	129/893	
Total	31	17056/817		

**= Significant a the 0.01 level

Variations in growth pattern and leaf morphology were apparent (*Fig. 1 F-G*). Most of regenerated plants had normal growth but a few dwarf variants (low growth rates) and 7 plants exhibited rapid growth with foliar modifications. Similar observation was reported in *Populus alba* L. x *P. grandidentata* MICHX. by SON et al. (1993). A dwarf variant was observed on Rosa hybrid L. cv. using somaclonal variation (ARENE et al., 1993). A few leafy structure plantlets (shoot formation with only leaves) were observed which did not produce root system and died in root induction medium. Morphology variation appeared on 40-month-old callus of *Populus nigra* (SUMIYAET et al., 1988). The phenotypic variation was reported in *Populus alba* cv. 5972-38 (Leuce section) using plant regeneration from somatic tissue (ANTONETTI and PINON, 1993). Somaclonal variants for qualitative and quantitative characters in higher plants (more than 50 species) were listed in different reviews (SCOWCROFT and LARKIN, 1982; EVANS and SHARP, 1986; AHLOOWALIA, 1986). New rice varieties have been developed using pollenhaploid somaclonal method (HESZKY et al., 1989). Somaclonal variation during interior spruce (*Picea glauca engelmannii* complex) somatic embryogenesis culture showed high degree of genetic stability in morphological behavior and was introduced as an appropriate *in vitro* culture system for clonal propagation of interior spruce (ESTMAN et al., 1991).

Chromosome counts on root segments of regenerants in our experiment showed to have diploid (38), aneuploid (21, 23 and 35) and polyploid (56) plants (*Fig. 1 D-F*). Five hundred and thirty-six *in vitro* plantlets were regenerated from long-term callus culture. At least 42 of the regenerates differed in some way from the original clones (7.8%). The frequency of induced variation (7.8%) in our experiment was higher than that which was reported on poplar from the sections Aigeiros and Tacamahaca (1.33%) by ANTONETTI and PINON (1993). These authors also observed variation on ploidy level (4n) in Leuce poplar callus after 10 subcultures.

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Buchbesprechungen

Gentechnik bei Pflanzen. Von F. und R. KEMPKEN. 2000. Springer Verlag, Berlin, Heidelberg, New York. ISBN 3-540-67547-7. 245 Seiten, 192 Seiten mit 18 Boxen, Abbildungen und Glossar.

Die Entstehungsgeschichte dieses Buches mutet gleichfalls glorifizierend wie die Aufrechterhaltung der Motivation bei der Durchführung des Projekts an. Wie die Autoren im Vorwort verraten, haben sie nach einer öffentlichen Diskussion über den Kenntnisstand der Bevölkerung zum Thema Gentechnik einer spontanen Eingebung folgend ihr Vorhaben an den Springer Verlag herangetragen. Sie wurden beflügelt von der Idee, dass der offensichtliche Mangel an Informationen zur Gentechnik, ihren Methoden und Zielen in der Bevölkerung behoben werden muss, damit die in Funk und Fernsehen sowie in Druckmedien gebotenen, teilweise sehr verkürzten, irreführenden oder falschen Behauptungen nicht die einzige Informationsquelle einer interessierten Bevölkerung bleiben. Es ist in der Tat schon besorgniserregend, wenn viele Deutsche trotz eines recht guten Bildungsniveaus der Meinung sind, dass Nahrungsmittel, die konventionell erzeugt wurden oder vom Bio-Bauer kommen, keine Gene enthalten und nur die Gentechnik für die krankmachenden Gene verantwortlich ist. Die Autoren sind somit berechtigterweise der Meinung, dass der schamlos betriebenen Verdummung der Menschen etwas entgegen gesetzt werden muss. Das vorliegende Buch, dass sich bewusst nicht an Spezialisten richtet, sondern für Lehrer, Schüler der gymnasialen Oberstufe sowie für Studenten der Naturwissenschaften und der Medizin geschrieben wurde, stellt ein sehr schönes Beispiel für ein gelungenes Umsetzen der anfänglichen Motivation dar. In verständlicher Sprache ist es den Autoren gelungen, verschiedene Aspekte der Erzeugung von Lebensmitteln als Ergebnis menschlicher Zuchtwahl, die wichtigsten Basismethoden der Gentechnik sowie Ziele und Beispiele gentechnischer Veränderungen zu beschreiben. Besonders hervorzuheben ist die umfassende Besprechung von Risikopotentialen, die bei der Anwendung gentechnischer Methoden resultieren können. Eine derart offene Auseinandersetzung mit dieser Thematik ist lobenswert, ist meines Erachtens aber auch für eine ehrliche und sachliche Diskussion essentiell. In einer weiteren Auflage, wenn es einmal dazu kommen sollte, werden die Diskussionen zu Nutzen und Risiken der Gentechnik bei Bäumen sicher auch ihre Beachtung finden.

M. FLADUNG (Grosshansdorf)

Bewertung von Umweltwirkungen von gentechnisch veränderten Organismen im Zusammenhang mit naturschutzbezogenen Fragestellungen. Berichte des Umweltbundesamtes, Band 3/01. Von M. LEMKE und G. WINTER. 2001. Erich Schmidt Verlag, Berlin. ISBN 3-503-06081-1. X und 373 Seiten. Kartoniert € 49,80 / sFr. 84,–.

Die Bewertung der Folgewirkungen des Ausbringens von gentechnisch veränderten Organismen (GVO) auf die Umwelt gestaltet sich aufgrund der Komplexität ökologischer Zusammenhänge und Wechselwirkungen sowie deren Eintrittswahrscheinlichkeit schwierig. Gegenstand des Berichts ist eine Fachtagung vom November 2000, auf der ein Gutachten über rechts- und naturwissenschaftliche Fragestellungen zum großflächigen Anbau transgener Kulturpflanzen diskutiert wurde.

Der Bericht gliedert sich in zwei Teile. Im ersten Teil ist das Gutachten abgedruckt (143 Seiten), in dem zunächst potentielle Folgewirkungen eines großflächigen Anbaus transgener Kulturpflanzen dargestellt sind. Ferner ist dargelegt, dass die vom Gentechnikgesetz geforderte Vertretbarkeit in Form der Risiko-Nutzen-Abwägung bei künftigen Genehmigungsverfahren zum Inverkehrbringen im Prozess der Risikobewertung verstärkt berücksichtigt wird. Weiterhin wird festgestellt, dass das geltende Gentechnikrecht nur bedingt zur Steuerung der mit dem großflächigen Anbau von GVO im Zusammenhang stehenden Risiken geeignet erscheint, und die Auffassung vertreten, dass die flächenbezogenen Instrumente des Naturschutzrechts auch nach erteilter Vermarktungsgenehmigung neue Perspektiven für ein Management der Risiken des großflächigen Anbaus transgener Kulturpflanzen bieten können.

Im zweiten Teil (224 S.) folgen 15 Beiträge und die Zusammenfassungen der Diskussionen der Fachtagung. Die Beiträge zeigen, dass das Instrument der Landschaftsplanung genutzt werden kann, um Gebiete auszuweisen, in denen der Anbau gentechnisch veränderter Organismen zu unterbleiben hat, und solche, in denen die Ausbringung transgener Kulturpflanzen aus naturschutzfachlicher Sicht unproblematisch erscheint. Im Laufe der Tagung hat sich herausgestellt, dass die Einrichtung eines Standortregisters und eines Genkonstruktregisters sowohl für die Gewährleistung der Rückverfolgbarkeit transgenem Erbgutes im Zusammenhang mit einem fallspezifischen Monitoring, als auch der allgemeinen Umwelt-