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KEIMVERSUCHE UND BIOMETRISCHE MESSUNGEN BEI EINIGEN *EPILOBIUM* – ARTEN SIEBENBÜRGENS

Martin KEUL*

Zusammenfassung: Die Samenkeimung bei *Epilobium hirsutum* und *E. parviflorum* wurde unter Laborbedingungen (22-24°C, Petri-Schalen, Filterpapier, Gartenerde) nach Belichtung (Weißlicht, spektraler Hellrot- und Dunkelrot-Bereich) bzw. im Dauerdunkel untersucht. *E. hirsutum*-Samen keimen im Licht und im Dauerdunkel, *E. parviflorum*-Samen dagegen nur im Weißlicht bzw. im Hellrot, nicht aber im Dauerdunkel und unter Dunkelrot-Belichtung. Die Samenkeimung bei *E. parviflorum* ist lichtabhängig und wird vom Photorezeptor Phytochrom gesteuert. Die in Gartenerde umgepflanzten Keimlinge überleben zu etwa 50 bis 60%, wachsen aber äußerst langsam. Biometrische Messungen zur quantitativen Erfassung morphologischer Merkmale (Pflanzenhöhe, oberirdische Masse, Anzahl Stängelknoten, Blatt- und Blütenblattgrößen) wurden an je 10 Pflanzen siebenbürgischer Populationen von *E. hirsutum, E. palustre, E. parviflorum, E.montanum, E.collinum, E. alsinifolium, E. nutans* und *Chamerion angustifolium* (=*Epilobium angustifolium*) durchgeführt.

Schlüsselworte: Epilobium, Samenkeimung, biometrische Messungen, Pflanzenwachstum.

Einleitung

Weidenröschen (*Epilobium*-Arten) werden in der traditionellen Volksmedizin seit lange als bewährte Mittel bei Frauen - und Männerleiden (insbesondere bei benigner Prostata-Hyperplasie, Harnblasen - und Nierenleiden, bei hormonalen Störungen oder als schmerzlinderndes Mittel, äußerlich zur Wundheilung u.a. (Tămaş, 1997; Stănescu et al., 2004; Buchwald et al., 2006) verwendet und meist als Infusionen und Tinkturen der oberirdischen krautigen Pflanzenteile (*Epilobii herba*) oder als Wurzelextrakte (*Epilobii radix*) verabreicht.

Die moderne Phytopharmazie hat viele der empirisch gesammelten Erfahrungen über die Heilwirkung bestimmter *Epilobium*-Arten wiederentdeckt, teilweise bestätigt und durch intensive Forschungen der letzten Jahre wissenschaftlich untermauert (vgl. Kujawski et al., 2011). Dabei wurden bisher ungenügend bekannte oder auch viele neue (antibakterielle, antivirale, antiproliferative, antitumorale, antioxidative, u.a.) Effekte bei einem breiten Spektrum von Arten der Gattungen *Epilobium* und *Chamerion* nachgewiesen, die umfangreiche taxonomische, ökologische, biochemische, genetische, molekularbiologische u.a. Untersuchungen zur Erweiterung unserer Kenntnisse

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hinsichlich ihrer biologischen Eigenschaften und ihrer besseren phytopharmazeutischen Nutzung anregten (Vălimăreanu und Deliu, 2008; Bejenaru et al., 2009; Ştef et al., 2009; Tămaş et al., 2009; Deliu et al., 2013; Stolarczyk et al., 2013;).

Erwähnt sei in diesem Zusammenhang der in den letzten Jahren gelungene experimentelle Nachweis zur Hemmung der Zellproliferation menschlicher Prostata-Epithelien *in vitro* durch alkoholische *Epilobium*-Extrakte (vgl. dazu Celeste, 2008). Im Tierversuch wurde gezeigt, dass die Verabreichung flavonoidreicher alkoholischer Extrakte der Arten *Epilobium hirsutum* L., *E. parviflorum* Schreb. und *Chamerion angustifolium* (L.) Holub (=syn. *Epilobium angustifolium* L.) keine cytotoxische Wirkungen auf lebenswichtige Organe ausübt (Roman et al., 2010).

Von den auf allen Kontinenten (außer der Antarktis) verbreiteten mehr als 165 Arten (bzw. über 185-200 Taxa) der Gattung *Epilobium* (*Onagraceae*), kommen in Europa 28 (Raven, 1980) und in Rumänien 14 Arten vor (Morariu, 1957; Ciocârlan, 2009). Außer diesen heute der Gattung *Epilobium* untergeordneten Arten ist noch das Schmalblättrige Weidenröschen [*Chamerion angustifolium* (L.) Holub] mit ähnlichen heilkräftigen Eigenschaften zu erwähnen, das derzeit mit anderen Arten der Gattung *Chamerion* Adans. angehört (Raven, 1980; Ciocârlan, 2009), aber in vielen rezenten Arbeiten (z. B. bei Celeste, 2008; Grainger und Turkington, 2012 u.a.) weiterhin als *Epilobium angustifolium* L. bezeichnet wird.

Im Rahmen eines multidisziplinären Forschungsprojektes zur potentiellen pharmazeutischen Verwertung der in Siebenbürgen verbreiteten *Epilobium*-Arten, wurden die Samenkeimung und das Keimlingswachstum bei *Epilobium hirsutum* und *E. parviflorum* unter Laborbedingungen untersucht und biometrische Messungen zur Erfassung artspezifischer morphologischer und wachstumsgebundener Merkmale bei *Epilobium*-Exemplaren natürlicher Populationen während der Blütezeit durchgeführt.

Material und Arbeitsmethoden

Untersuchungsmaterial. Als Pflanzenmaterial wurden verwendet: 1. Samen von *Epilobium hirsutum* L. und *E. parviflorum* (Schreb.) aus der Samenkollektion des Botanischen Gartens „Alexandru Borza" Cluj-Napoca zwecks Durchführung von Keimversuchen; 2. aus Samen regenerierte Keimpflanzen der Arten *E. hirsutum* und *E. parviflorum* zur Untersuchung ihres Wachstums in den frühen Wachstums- und Entwicklungsphasen; 3. während der Blütezeit aus natürlichen *Epilobium*-Populationen Siebenbürgens geerntete Exemplare der Weidenröschenarten *Epilobium hirsutum* L. (Vălişoara-Colţesti-Aiud); *E. palustre* L. (Şesuri-Maramureş); *E. montanum* L. (Valea Ierii und Poiana Horea); *E. nutans* F.W. Schmidt (Muntele Mare); *E. alsinifolium* Vill. (Muntele Mare) und *Chamerion angustifolium* (L.) Holub=syn. *Epilobium angustifolium* L. (Arieşeni) für biometrische Messungen.

Keimversuche wurden mit *Epilobium hirsutum* - und *E. parviflorum*-Samen 6, 9 und 12 Monate nach der Ernte und trockener Lagerung bei 4° C durchgeführt. Die Versuche wurden in Petri-Schalen auf einer mit Leitungswasser befeuchteten Unterlage (Filterpapier bzw. Gartenerde+gewaschener Flusssand, 1:1) angesetzt. Die Keimung erfolgte unter Laborbedingungen bei 22-24° C, im Licht (L) bzw. im Dunkeln (D). Für die im Licht gekeimten Proben wurde diffuses Tageslicht bei natürlichem Tag - und Nachtwechsel verwendet und durch künstliches Zusatzlicht mit Fluoreszenzröhren 8 Stunden pro Tag entspechend einer Photonenstromdichte von etwa 1.000 μmoli/cm^2.s ergänzt.

Für die Keimversuche im hellroten Spektralbereich (HR= *engl.* red light bzw. r) wurde eine Kombination von Fluoreszenzröhren und roter Celophan-Folie mit spektraler Durchlässigkeit bei ca. 600-675 nm verwendet, während die Versuche im dunkelroten Spektralbereich (DR= *engl.* far red light bzw. fr) mit Glühlampenlicht von 75 W und einer davorgeschalteten Kombination aus blauem Plexiglas und roter Plastikfolie mit spektraler Durchlässigkeit von über 700 nm durchgeführt wurden.

Der Ablauf der Samenkeimung wurde täglich über eine Dauer von 20 Tagen nach dem Ansetzen in je 3 Parallelversuchen verfolgt.

Untersuchung des Keimlingswachstums. Nach Abschluss der Keimversuche wurden die Keimlinge von *Epilobium hirsutum* und *E. parviflorum* in Blumentöpfe (Gartenerde und Flusssand, 1:1) umgesetzt und im Labor (22-24 °C, diffuses Tageslicht bei natürlichem Licht-Dunkelwechsel und 8stündigem Zusatzlicht/Tag mit Fluoreszenzröhren) kultiviert.

Biometrische Messungen hinsichtlich Pflanzenhöhe, Stängelmasse, Anzahl der Stängelknoten, Länge der Internodien, Blatt- und Blütenblattdimensionen u.a. Parameter wurden an je 10 (ausnahmsweise an 5 oder 20) Pflanzenexemplaren der untersuchten Arten während der Blütezeit durchgeführt. Die Angaben wurden statistisch verarbeitet. Neben dem Mittelwert und der Standardabweichung (m±s) ist zum Vergleich der Variation des jeweiligen Merkmals auch der Variationskoeffizient (CV%=s.100/m) und die Variationsbreite (minimaler-maximaler Wert) angegeben. Der Wachstumsverlauf (das Wachstumsmuster) des oberirdischen krautigen Stängels wurde als kumulierter Längenwert der aufeinanderfolgenden Internodien in % der erreichten Endlänge (Gesamtlänge) in Anlehnung an Richards (1965) berechnet.

Ergebnisse und Diskussion

Keimversuche. Eingehendere Untersuchungen über die Samenkeimung bei verschiedenen *Epilobium*-Arten wurden besonders von Myerscough und Whitehead (1966) bei *Epilobium adenocaulon* Hausskn. (=syn. *E. ciliatum* Raf.) und *E. montanum* L., sowie von Shamsi und Whitehead (1974a), Etherington (1983), Pérez-Fernández et al. (2006) u.a. bei *Epilobium hirsutum* L. in Verbindung mit verschiedenen physiologischen und ökologischen Fragestellungen durchgeführt. Die meisten Angaben über die Samenkeimung

wurden jedoch für die verwandte Art *Chamerion angustifolium* (L.) Holub (=syn. *Epilobium angustifolium* L.) erarbeitet (Myerscough und Whitehead, 1966; Giannini, 1972; Ruwet, 1975, Romme et al., 1995; Buchwald et al., 2006; Grainger und Turkington, 2012 u.a.), denn diese Art ist nicht nur als Heilpflanze, sondern auch dank ihrer Bedeutung als Pionierpflanze und ihrer häufigen Beteiligung am Aufbau vieler zirkumpolarer Pflanzengesellschaften der nördlichen Halbkugel (Myerscough, 1980) von Interesse.

Zusammenfassend ist aus diesen Versuchen ersichtlich, dass *Epilobium*- und *Chamerion angustifolium*-Samen allgemein non-dormant sind und schon kurz nach ihrer Ernte in wenigen Tagen innerhalb eines breiten Temperaturbereiches (4-30 °C) keimen, wobei Licht (Shamsi und Whitehead, 1974a) und variable Temperaturen optimale Voraussetzungen für eine hohe Keimrate (40-80%) in den ersten 2 Jahren nach der Ernte darstellen, während die Keimfähigkeit bei älteren Samen rasch abnimmt und nach etwa 7 Jahren verlorengeht (Buchwald et al., 2006).

Die Keimrate der Samen zeigt dabei oft erhebliche Differenzen bei verschiedenen Populationen derselben Art, die durch die Lagerbedingungen (Lagerdauer, Temperatur, Feuchtigkeit usw.), die Keimmethode und die Keimbedingungen (Baskin und Baskin, 2001), sowie durch die meteorologischen Verhältnisse während der Samenreife (Buchwald et al., 2006) und anderen Faktoren bedingt werden. So geben z. B. Treberg und Turkington (2008) sowie Grainger und Turkington (2012) für *Chamerion angustifolium*-Bestände auf demselben Areal Keimraten von etwa 23% an, während Romme *et al.* (1995) und Granstrom (1987) in anderen Populationen Keimwerte von 66% bzw. sogar von 89% erhalten.

Vorliegende Ergebnisse zur Samenkeimung bei *Epilobium hirsutum* und *E. parviflorum* variieren nach Substrat (Filterpapier, Gartenerde), Belichtung (Licht oder Dunkel), Spektralbereich (HR bzw. DR), Lagerdauer u. a.

Tabelle 1. Der Verlauf der Samenkeimung (m±s, CV%) bei *Epilobium hirsutum* L., 6 Monate nach der Ernte, auf Filterpapier bzw. auf oder in Gartenerde, im Licht (L.) und im Dauerdunkel (D).

Ansatz	Tage nach dem Ansetzen			
	4	8	12	16
L Filterpapier	62,0±6,0 (9,7%)	70,0±2,0 (2,9%)	74,0±2,0 (2,7%)	76,0±1,0 (1,3%)
D Filterpapier	10,0±2,0 (20,0%)	26,00±2,0 (7,7%)	64,00±4,0 (6,3%)	64,00±4,0 (6,3%)
L auf Erde	38,0±2,0 (5,30%)	86,0±2,0 (2,3%)	87,0±1,0 (1,1%)	87,0±1,0 (1,1%)
D in Erde	0±0,0 (0,0%)	8±0,0 (12,5%)	10,0±0,0 (20,0%)	12,0±0,0 (33,0%)

Die in Tabelle 1 zusammengefassten Ergebnisse zum Ablauf der Keimung bei *Epilobium hirsutum*–Samen im Alter von 6 Monaten nach der Ernte und Lagerung bei 4 °C zeigen, dass die Keimung im L am 4. Tag nach dem Ansetzen sowohl auf Filterpapier (FP) als auch auf Gartenerde (E) mit hohen Keimwerten (62% bzw. 38%) beginnt, danach sigmoid zunimmt und am 12. Tag praktisch abgeschlossen ist, wobei am 16. Tag nach der Aussaat (Tabelle 1) sowohl im L als auch im D relativ hohe Endwerte von 64% (D, FP), 76% (L, FP) bzw. 87% (L, E) erreicht werden.

Diese Versuche zeigen, dass Belichtung und die Substratnatur die Keimung bei *Epilobium hirsutum* relativ wenig beeinflussen, außer dass die Keimung auf FP unter Licht rascher und mit hohem Keimwert beginnt (62% am 4. Tag), im D jedoch verzögert wird und kleinere Endwerte (64%) erreicht werden.

Der Einfluss der Natur der Unterlage ist im L und im D unterschiedlich. Im L verläuft die Keimung auf E langsamer, erreicht aber höhere Endwerte (87%) als auf FP (76%). Bei den in die Erde ausgesäten Samen (D) wird die Keimung bis zum 8. Tag nach der Aussaat verzögert und die Endwerte erreichen kaum 12%. Dagegen keimen die Samen auf E sowohl unter L als auch im D besser als auf FP.

Die Daten aus Tabelle 1 führen zur Schlußfolgerung, dass die Samenkeimung bei *Epilobium hirsutum* relativ lichtunabhängig ist und dass die Samen sowohl bei Belichtung als auch im Dunkeln keimen. Bei Belichtung wird die Keimung im Vergleich zu den Dunkelproben stark beschleunigt und allgemein gefördert, während die Keimprozesse im D verzögert und in gewissem Ausmaß gehemmt werden.

Die im gleichen Versuchsansatz untersuchte Samenkeimung bei *Epilobium parviflora* ergab die Ergebnisse aus Tabelle 2.

Tabelle 2. Die Keimraten bei *Epilobium parviflorum* Schreb., 6 Monate nach der Ernte, auf Filterpapier bzw. auf oder in Gartenerde, im Licht (L.) und im Dauerdunkel (D).

Ansatz	Tage nach dem Ansetzen			
	4	8	12	16
L Filterpapier	60,0±8,0 (13,3%)	68,0±8,0 (11,8%)	72,0±8,0 (11,8%)	76,0±8,0 (10,5%)
D Filterpapier	4,0±0,0 (0,0%)	4,0±0,0 (0.0%)	6,0±2,0 (33,3%)	6,0±2,0 (33,3%)
L auf Erde	48,00±4,0 (8,3%)	80,0±12,0 (15,0%)	82,0±10,0 (12,2%)	84,0±8,0 (9,5%)
L in Erde	26,00±2,0 (7,7%)	40,0±8,0 (20,0%)	47,00±7,0 (14,9%)	54,0±6,0 (11,1%)
D auf/in Erde	0±0,0 (0,0%)	0±0,0 (0,0%)	0±0,0 (0,0%)	0±0,0 (0,0%)

Bei *Epilobium parviflorum* induziert die Belichtung der Samen sowohl auf FP (76%) als auch auf E (84%) relativ hohe Werte, wobei auch die von einer dünnen Erdschicht bedeckten Samen bei L Keimwerte von bis zu 54% aufweisen. Dagegen werden im D auf FP maximal 6% Keimprozente erreicht, während die Keimung im D bei den auf die Erde bzw. in die Erde ausgesäten und von einer dünnen Erdschicht bedeckten Samen ganz unterbleibt (0%).

Aus diesen Ergebnissen ist zu schließen, dass die Samenkeimung bei *Epilobium parviflorum* nur im Licht stattfindet, die Samen also als photoblastisch (Lichtkeimer) angesehen werden müssen.

Diese Versuche belegen somit, dass die Samenkeimung bei *Epilobium hirsutum* und *E. parviflorum* verschiedene ökologische Lichtansprüche aufweist.

Diese Schlussfolgerung steht dabei nur scheinbar im Widerspruch zu den Ergebnissen der 2. Variante aus Tabelle 2, bei der unter Belichtung relativ hohe Keimraten von 26 bis 54% auch bei den unter eine dünne Erdschicht ausgesäten Samen erreicht werden, die gegen direkten Lichteinfall durch die Bodenpartikel abgeschirmt sind. In diesem Fall widerspiegeln die erhaltenen Keimergebnisse das in der betreffenden Tiefe der Erdschicht erreichte Ausmaß der Belichtungsintensität (Myerscough und Whitehead, 1966).

Samen, die ebensogut im Licht und bei Dunkelheit keimen, sind nach Bliss (1971) oft solche, die bei Frühjahrsüberschwemmungen von Schwemmmaterial (Sand, Schlamm) bedeckt werden, im Gegensatz zu Lichtkeimern, deren Samen selten vergraben werden. *Epilobium hirsutum* bevorzugt feuchte und nasse Habitate (Moore, Flussufer usw.) und die Samenkeimung ist nur teilweise von Licht abhängig (Shamsi und Whitehead, 1974a; King, 1975), während *E. parviflorum* offene, bodenfeuchte Standorte an Gewässern u.a. besiedelt, die Keimung bei dieser Art als lichtabhängig gilt und temperaturempfindlich ist (Lehmann, 1912; Taylorson und Hendricks 1972 u.a.) und dabei mit hohen Keimwerten (90%) auch unter Wasser (also normalerweise bei Licht) stattfindet (Brandes und Evers, 1999).

In folgenden Versuchen wurde die spektrale Abhängigkeit der Samenkeimung beider Arten vergleichend überprüft, um die Beteiligung von Phytochrom (P) an der Induktion der Keimung nachzuweisen.

Der theoretische Hintergrund für die Durchführung dieser Keimversuche sei hier kurz umrissen:

Für die lichtabhängige Induktion und für den Ablauf bzw. die Steuerung vieler Pflanzenreaktionen durch Licht, wie z. B. bei der Samen - und Farnsporen-Keimung (Keul et al., 1993), der Photomorphogenese u.a., ist nach heutigen Erkenntnissen das Phytochrom-Molekül (P) als Photorezeptor beteiligt (Furuya, 1993).

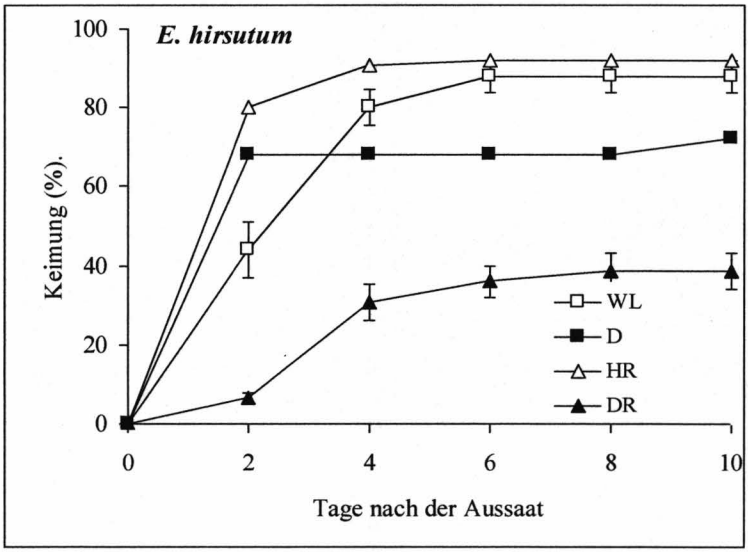

Abb. 1. Der Ablauf der Samenkeimung bei *Epilobium hirsutum* L., 8 Monate nach der Ernte, unter hellrotem (HR) und dunkelrotem (DR) Licht im Vergleich zu den Weißlicht (WL)- und Dunkel (D)-Kontrollen.

Phytochrom kommt in physiologisch aktiven und physiologisch inaktiven Formen vor, die sich u.a. durch unterschiedliche Absorptionsmaxima unterscheiden und nach spezifischer Lichtabsorption im roten Spektralbereich reversibel ineinander umgewandelt werden. Das physiologisch aktive P, mit einem Absorptionsmaximum um 720 nm im DR-Bereich (*engl.* far red, daher als Pfr bezeichnet), wird durch Absorption von Lichtquanten dieser Wellenlänge reversibel in die physiologisch inaktive Form Pr mit maximaler Absorption im hellroten (HR)-Bereich bei 660 nm (*eng.* red, daher als Pr) umgewandelt. In lichtabhängigen, Phytochrom-gesteuerten Prozessen, wird bei natürlicher Belichtung ein gewisses Konzentrationsverhältnis zwischen physiologisch aktivem Pfr und physiologisch inaktivem Pr eingestellt, wodurch diese Vorgänge induziert und gesteuert werden (Briggs und Olney, 2001).

Die in folgenden Versuchen erzielten Angaben hinsichtlich der Bedeutung von Licht für die Induktion der Samenkeimung und ihre spektrale Abhängigkeit im Falle der untersuchten *Epilobium-Arten* konnten die Beteiligung des Photorezeptors Phytochrom für die Induktion der Keimung bei *E. parviflora*-Samen experimentell belegen (Abb. 1 und 2).

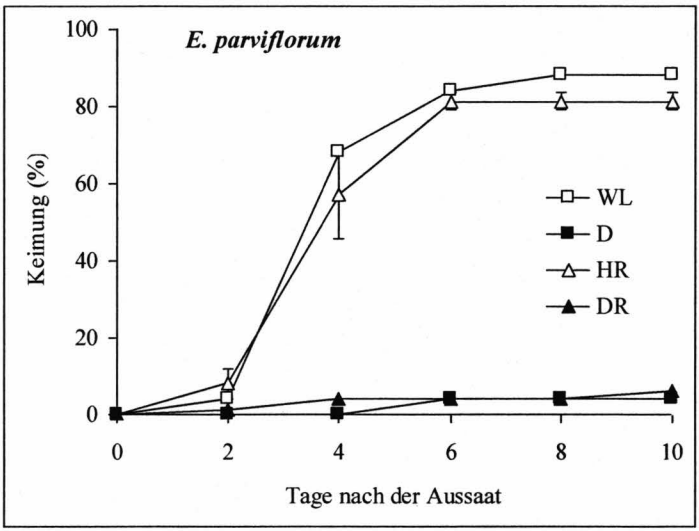

Abb. 2. Der Ablauf der Samenkeimung bei *Epilobium parviflorum* Schreb., 8 Monate nach der Ernte, unter hellrotem (HR) und dunkelrotem (DR) Licht im Vergleich zu den Weißlicht (WL) - und Dunkel (D)-Kontrollen.

Aus diesen Ergebnissen ist ersichtlich (Abb. 1), dass die Samenkeimung bei *Epilobium hirsutum* auch im Dunkeln (D) bzw im DR-Licht ziemlich hohe Werte erreicht, wobei Weißlicht (WL) und HR die Keimrate erwartungsgemäß stimulieren. Dagegen werden bei *E. parviflorum* (Abb. 2) nur unter WL und HR hohe Keimraten erreicht, während die Keimung im Dunkeln (D) oder unter DR-Licht (720 nm) nahezu blockiert wird; erst bei der auf D folgenden Nachbelichtung (WL oder HR-660 nm) wird die Keimung wie in Abb. 2 unmittelbar induziert (Ergebnisse nicht dargestellt).

Diese Versuche zeigen, dass die beiden *Epilobium*-Arten in Bezug auf die Samenkeimung verschiedene Lichtbedürfnisse aufweisen, wobei die Keimung bei *E. parviflorum* gegenüber *Epilobium hirsutum*-Samen, die auch in Abwesenheit von Licht keimen (Abb. 1), strenger lichtabhängig ist. Unter natürlichen Bedingungen keimen *Epilobium*-Samen nach ihrem Abfall schon im Herbst bzw. Winter, sofern sie nicht von Schwemmmaterial bedeckt werden und somit in die Diasporenbank gelangen (Grime, 1981).

Keimlingswachstum. Die aus Samen regenerierten Keimlinge von *Epilobium hirsutum* und *E. parviflorum* wurden zur Untersuchung ihres weiteren Wachstums in Blumentöpfe auf Gartenerde umgepflanzt und im Labor bei 22-24°C, diffusem Tageslicht und zusätzlichem Fluoreszlicht (8 Std/Tag) weiterkultiviert.

Die Überlebensrate der Keimpflanzen beträgt etwa 50-60%, wobei Wachstum und Entwicklung äußerst langsam verlaufen. Längenmessungen des Stängelchens von je 5 Pflänzchen zeigen 5 Monaten nach ihrer Umsetzung in

Blumentöpfe 14±2,6 cm Höhe und 9±1,6 Blättchenpaare bei *Epilobium hirsutum*, bzw. 7,8±0,84 cm Länge und 12,6±0,9 Blättchenpaare bei *E. parviflorum*. Die frühjährliche Regeneration der *Epilobium*-Bestände scheint daher offensichtlich durch das kräftige vegetative Austreiben unterirdischer Stängel zu erfolgen (Bliss, 1971; Shamsi und Whitehead, 1974b), doch sollte nach Welling (2002) die Bedeutung der Regeneration durch Samen aus der Diasporenbank nicht unterschätzt werden.

Biometrische Messungen. In Tabelle 3 sind einige biometrische Angaben der untersuchten *Epilobium*-Arten hinsichtlich ihrer mittleren Stängellänge und ihrer Blattgrößen zusammengefasst. Weitere in der Tabelle nicht angeführte Messangaben beziehen sich auf die Stängelmasse, die Größe der Blütenblätter und andere Parameter.

Tabelle 3. Biometrische Angaben (arithm. Mittelwert und Standardabweichung, Variationskoeffizient in %, Variationsbreite) hinsichtlich der mittleren Pflanzenhöhe (cm) und der Dimensionen (Länge, Breite) der mittleren Stängelblätter von *Epilobium*-Arten siebenbürgischer Populationen.

Art	Standort	Pflanzenhöhe cm	Blattgröße (cm)	
			Länge	Breite
E.hirsutum	Vălişoara Colţesti Aiud	76,4±6,2 (8,2%) (68-87)	7,0±0,7 (10%) (5,5-8)	1,4±0,2 (14%) (1-1,9)
E. montanum	Valea Ierii	89.7±17,5(19%) (68-126)	3,8±0,7 (18%) (3,1-5,1)	1,3±0,3 (23%) (0,8-1,6)
	Poiana Horea	89,1±35,0(40%) (45-138)	3,5±1,0 (29%) (2-5,8)	0,9±0,3 (33%) 0,5-1.5
E. palustre	Şesuri Maramureş	49,8±4,6 (9,2%) (9,2%)	2,8±0,6 (22%) (2-4,1)	1,2±0,3 (21%) (0,8-1,6)
E. nutans	Muntele Mare	15,0±2,8 (19%) (11-20)	1,9±0,5 (26%) (1-3)	0,5±0.2 (40%) 0,2-0,8
E. alsinifolium	Muntele Mare	12,6±2,3 (18%) (9-15)	2,5±0,3 (12%) (2-3)	1,0±0,1 (10%) (0,8-1,3)
E. angustifolium	Arieşeni	118±10,5 (9%) (103-125)	8,8±1,6 (18%)* (7-12)	1,3±0,3(23%)* (1-1,5)

*obere Blätter unterhalb des Blütenstandes

Die von uns erzielten Angaben stimmen im allgemeinen mit den in der Flora Rumäniens (Fl. Rum.) für *Epilobium hirsutum* L. angegebenen Größenwerten (Stängellänge 50-150 cm, Blattlänge 4-12 cm, Blattbreite bis 3 cm) überein (Morariu, 1957). Nach unseren Untersuchungen ist der krautige Stängel mit einer mittleren Masse von 65,2±13,9 g (51-90 g) zur Blütezeit in 11-18 Internodien gegliedert, deren Verteilung vom Stängelgrund in Richtung Stängelspitze in Abb. 3 dargestellt ist. Daraus geht hervor, dass die längsten, im Stängelwachstum ältesten Internodien am Stängelgrund lokalisiert sind, die kürzesten und jüngsten Internodien dagegen zur Stängelspitze hin erscheinen. Die Blattwerte (vom 5. Knoten aufwärts gemessen) sind in Tabelle 3 enthalten. Die Blütenblattgröße (in Tabelle 3 nicht angegeben) reicht von 0,8-1,0 cm Länge und 2-3 mm Breite bei Colţeşti Vălişoara bis 1,4-1,8 cm Länge und 1-1,6 cm Breite bei Şăulia, zeigt demnach je nach Bestand eine große Variabilität.

Die statistische Verteilung der Internodienlängen (cm) des Stängels ist bei *Epilobium hirsutum* L. und *E. palustre* L. in Abb. 3 dargestellt.

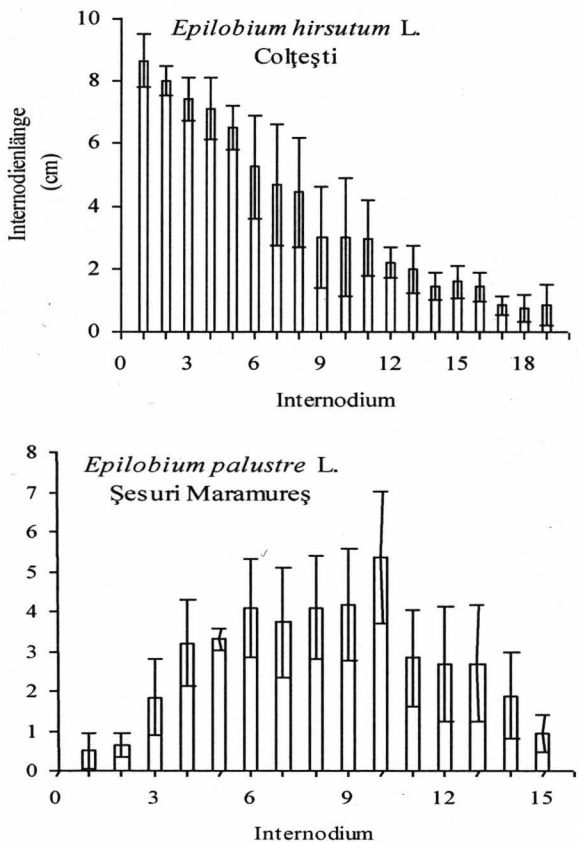

Abb. 3. Die Verteilung der mittleren Länge (cm) der in Richtung Stängelspitze aufeinanderfolgenden Stängelinternodien bei *Epilobium hirsutum* L. (Colţeşti) und *Epilobium palustre* L. (Şesuri-Maramureş).

Epilobium montanum L. (Berg-Weidenröschen) wurde in den Populationen von Valea Ierii und Poiana Horea untersucht. Die Pflanzen bei Valea Ierii erreichen zwischen 67,5 und 126 cm Höhe (89,7±17,5 cm, CV 19,5 %) mit 13-32 (24,3±6,45) Internodien (Abb. 4), die sehr variable mittlere Masse des krautigen Teils beträgt 2,5-11g (4,9±2,2 g), die mittleren Stängelblätter sind 4,4-7,3 cm (5,8±1,0 cm) lang und 1,8-3,3 cm (2,4±0,5 cm) breit, der Blattstiel 1-3 mm (2,1±0,6 mm), die oberen Blätter sind 3,1-5,1 cm (3,8± 0,7 cm) lang und 0,8-1,6 cm (1,3±0,3 cm).

Die biometrischen Angaben für die Population bei Poiana Horea sind: Höhe 45-138 cm (Mittel 89,1±35 cm), Masse 13-86 g (36,4±24,4 g), untere Blätter 6,4-10,8 cm (7,4±1,3 cm) lang, 1,6-3,4 cm (2,2±0,5 cm) breit, kurz gestielt (-15 mm) , mittlere Blätter 4,4-10,5 cm (7,0±1,8 cm) lang, 1-3,4 cm (2,0±0,6 cm), Blattstiel 2-13 mm, obere Blätter 2-5,8 cm (3,5±1,0 cm) lang und 0,5-1,5 cm (0,9±0,3 cm) breit. Nach der Fl. Rum. variiert die Pflanzenhöhe zwischen 10 und 80 (100) cm, die Blattlänge von 3 bis 10 cm, die Blattbreite von 1,5 zu 5 cm.

Epilobium palustre L. (Sumpf-Weidenröschen) bei Şesuri (Maramureş) hat eine mittlere Stängelhöhe von 43-56 cm (49,8±4,6 cm) mit 10-15 Internodien (Abb. 3), eine Masse von 0,4-0,7 g (0,5±0,1), mittlere Blätter von 3,2-5 cm (4,2±0,6 cm) Länge, 1,2-1,8 cm (1,5±0,2 cm) Breite, obere Blätter 2,0-4,1 cm (2,8±0,6) Länge und 0,8-1,6 cm (1,2±0,3) Breite de 5,61±1,14 cm. Aus Abb. 3 ist ersichtlich, dass der Stängel bei *Epilobium palustre* in Bezug auf die Länge der aufeinanderfolgenden Internodien völlig anders gegliedert ist als bei *Epilobium hirsutum*, wobei die Verteilung der Internodienlänge glockenförmig gestaltet und die größten Längen im mittleren Abschnitt des Stängels lokalisiert sind. Aus den Längenmessungen der aufeinanderfolgenden Internodien wurden die Wachstumsmuster des Stängels bei *Epilobium hirsutum* L., *E. montanum* L., und *E. palustre* L. als kumulierte Internodienlängen in % der erreichten Endlänge in Anlehnung an Richards (1962) berechnet und für die genannten Arten in Abb. 4 vergleichend für dargestellt.

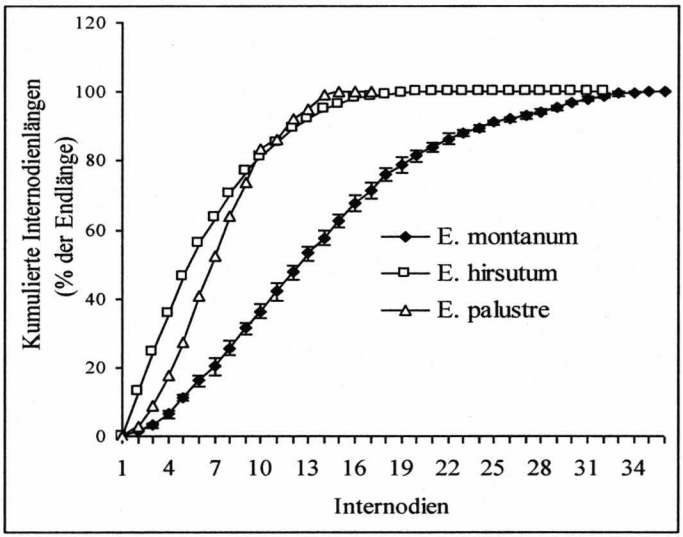

Abb. 4. Das Wachstumsmuster für *Epilobium montanum, E. hirsutum.* und *E. palustre*, dargestellt als kumulierte Internodienlängen (in % der Endlänge).

Die am Muntele Mare eingesammelten Exemplare von *Epilobium nutans* F.W. Schmidt (Nickendes Weidenröschen), mit 0-3 (4) dünnen Ausläufern am Stängelgrund, erreichen 11,5-20 cm (15,0±2,8 cm) Höhe; die Stängelmasse beträgt 0,2-0,47 g (0,4±0,1 g), die Länge der unteren, gestielten (1-3 mm) Blätter 4,4-7,3 cm (5,8±1,0 cm) und die Breite 1,8-3,3 cm (2,65±0,5 cm); die oberen Blätter im Blütenstand sind 1,1-3,0 cm (1,9±0,5 cm) lang und 2-8 mm (4,8±1,5 mm) breit.

Epilobium alsinifolium Vill. (Mieren-Weidenröschen), Muntele Mare, haben eine Höhe von 11,5 cm (12,6±2,3 cm) und eine Masse von ca. 2,75 g; die mittleren Blätter haben 2,3-4,0 cm (3,1±0,5 cm) Länge und 1-1,7 cm (1,2±0,2 cm) Breite, die oberen (unmittelbar unter dem Blütenstand) 2-3 cm Länge (2,5±0,3 cm) und 0,8-1,3 cm Breite (in der Fl. Rum. wird eine Höhe von 10-25 cm mit ca. 3 cm langen und etwa 1,5 cm breiten Stängelblättern angegeben).

Chamerion angustifolium (L.) Holub (=*Epilobium angustifolium* L.) aus der Population von Arieşeni, erreicht nach unseren Messungen eine Höhe von 100-125 cm (118,5±10,5 cm), einschließlich der Blütenstand von 28-40 cm (32,5±5,4 cm) Länge; die Masse des beblätterten Stängels beträgt 25-78 g (57,0±25,1 g), die unteren gestielten (0,5-1 cm) Blätter sind 14-16 cm (14,7±0,7 cm) lang und 1,4-2,5 cm (2,0±0,5 cm) breit, die oberen Blätter im Blütenstand 7-11 cm (8,8±1,6) lang und zwischen 1 und 1,5 cm (1,3±0,3 cm) breit.

Schlussfolgerungen

Die Samenkeimung von *Epilobium hirsutum* L. und *E. parviflorum* Schreb. wurde unter Laborbedingungen (22-24°C, Petri-Schalen, Filterpapier,

Gartenerde) nach Belichtung (Weißlicht, spektraler Hellrot- und Dunkelrot-Bereich) bzw. im Dauerdunkel untersucht.

Die durchgeführten Keimversuche zeigen, dass die beiden *Epilobium*-Arten in Bezug auf die Samenkeimung verschiedene Lichtbedürfnisse aufweisen. Bei *Epilobium hirsutum* werden sowohl im Licht als auch im Dauerdunkel ziemlich hohe Keimraten (70-80%) erreicht, was darauf hinweist, dass die Induktion der Keimung bei dieser Art relativ lichtunabhängig ist, während *E. parviflorum*-Samen nur im Weißlicht bzw. unter hellroter Belichtung (HR, ca. 650-670 nm), nicht aber im Dauerdunkel und unter Dunkelrot-Belichtung (DR, ca. 700-739 nm) keimen. Die Versuche führen zur Schlussfolgerung, dass die Induktion der Samenkeimung bei *E. parviflorum* gegenüber *E. hirsutum* streng lichtabhängig ist und vom Photorezeptor Phytochrom gesteuert wird.

Die auf Gartenerde umgepflanzten Keimlinge von *Epilobium hirsutum* und *E. parviflorum* überleben zu etwa 50 bis 60%, wachsen aber äußerst langsam. Fünf Monaten nach ihrer Weiterkultur auf Gartenerde erreichen die Pflänzchen im Mittel 14±2,6 cm mit 9±1,6 Blättchenpaaren bei *E. hirsutum* bzw. 7,8±0,84 cm Länge mit 12,6±0,9 Blättchenpaaren bei *E. parviflorum.*

Zur quantitativen Erfassung morphologischer Merkmale wurden biometrische Messungen an je (5)10 (-20) Pflanzenexemplaren siebenbürgischer Populationen von *E. hirsutum, E. palustre, E. parviflorum, E.montanum, E.collinum, E. alsinifolium, E. nutans* und *Chamerion angustifolium* (=*Epilobium angustifolium*) hinsichtlich Pflanzenhöhe, Stängelmasse, Anzahl der Stängelknoten bzw. der Internodien, Blatt- und Blütenblattgrößen) durchgeführt und das Wachstumsmuster für den Stängel bei *E. montanum*, *E. hirsutum* und *E. palustre* dargestellt.

Rezumat

S-a urmărit germinaţia seminţelor de *E. hirsutum* L. şi *E. parviflorum* Schreb. la 6, 9 şi 12 luni după recoltare şi stocare uscată la +4° C, în dependenţă de natura substratului de germinare (cutii Petri pe hârtie de filtru umectată, ghivece cu sol de grădină în amestec 1:1 cu nisip de râu spălat) şi condiţiile de iluminare (lumină albă sau la întuneric, respectiv în lumină spectrală de culoare roşu deschis (red light, cca. 650-670 nm) comparativ cu lumină spectrală din zona de culoare roşu îndepărtat (far red light, 700-730 nm).

Experienţele de germinare s-au efectuat în condiţii de laborator la 22-24° C. Pentru probele germinate la lumină, iluminarea s-a efectuat la lumina diurnă difuză din laborator în condiţiile alternanţei naturale dintre zi şi noapte suplimentată 8 ore pe timpul zilei cu tuburi fluorescente la un flux luminos total de cca. 1.000 μmoli/cm^2.s.

Rezultatele experienţelor arată că germinaţia seminţelor celor două specii de *Epilobium* analizate prezintă cerinţe diferite faţă de iluminare. Astfel, seminţele de *E. hirsutum* L. germinează în condiţii bune atât la lumină, cât şi la întuneric, lumina având cel mult un rol stimulator, iluminarea nefiind însă obligatorie pentru inducerea germinaţiei. În schimb, seminţele de *E. parviflorum* germinează numai la lumină (integrală albă şi în zona spectrală roşu deschis corespunzător absorbţiei maxime a

fitocromului Pr la 660 nm), germinaţia fiind practic blocată la întuneric şi în lumină spectrală din zona roşu închis (corespunzător absorbţie maximă a fitocromului Pfr la 720 nm). Rezultatele obţinute denotă că inducerea germinaţiei seminţelor fotoblastice de *E. parviflorum* Schreb. este controlată de sistemul de pigmenţi al fitocromului (P).

Plantulele de *E. hirsutum* L. şi *E. parviflorum* Schreb. regenerate din seminţe şi transplantate în ghivece pe sol de grădină cu nisip de râu arată că, în condiţii de laborator (ca. 25-28° C, iluminare cu tuburi fluorescente), supravieţuirea este de cca. 50-60%, creşterea plantulelor fiind extrem de înceată; astfel, după 5 luni de cultivare, tulpiniţa de *Epilobium hirsutum* atinge în medie o înălţime de 14±2,6 cm cu formarea a 9±1,6 perechi de frunzuliţe, iar la *E. parviflorum* o înălţime de 7,8±0,84 cm şi 12,6±0,9 perechi de frunzuliţe.

Măsurătorile biometrice şi de creştere efectuate la *Epilobium hirsutum* L., *E. parviflorum* Schreb., *E. montanum* L., *E. palustre* L., *E. nutans* F.W. Schmidt, *E. alsinifolium* Vill. şi *Chamerion angustifolium* (L.) Holub din populaţii identificate în Transilvania aduc precizări privind anumiţi indici morfologici şi variabilitatea lor (înălţimea şi masa tulpinii, numărul de internodii tulpinale, dimensiunile frunzelor şi petalelor) şi au permis redarea modelului de creştere specific pentru *Epilobium montanum*, *E. hirsutum* şi *E. palustre*.

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ECOLOGY OF *Armeria maritima* (Willd) ssp. *maritima* FROM BLĂJENII DE JOS, BISTRIŢA-NĂSĂUD COUNTY - A 2013 UPDATE OVERVIEW

Vlad Alexandru TOMA*, Claudia Simona LADAR**

Abstract: *Armeria maritima* (Willd) ssp. *maritima* is a halophile plant that grows in Bistriţa-Năsăud county, in the village Blăjenii de Jos, in a place called "La Sărătură". According to specialized literature, *Armeria maritima* (Willd) ssp. *maritima* grows in Romania only in Blăjenii de Jos. The species grows on 5 ha, but in time, the number of individuals decreased in the place monitored and restricted and increased in the peripherial places. This study was developed in a period of time between 2008 and 2013 and demonstrates that the human protective action in Nature is not good every time and also presents some aspects about *Armeria maritima* (Willd) ssp. *maritima* anatomy, morphology and physiology.

Keywords: *Armeria maritima* (Willd) ssp. *maritima*, Blăjenii de Jos, number of individuals, morphoanatomy, ecophysiology.

Introduction

The place "La Sărătură" is located in N-E of Transylvania, in Bistriţa-Năsăud county, on the territory of Blăjenii de Jos village. "La Sărătură" is a salty place where live some species that are typical for the salty soil, for example: *Salsola soda*, *Salicornia herabceea*, *Tamarix tetrandra*, *Limonium gmelini* and *Armeria maritima* (Willd) ssp. *maritima* (Chintăuan, 2000). There are a few issues related to this last species. First issue that occurs is the origin and perpetuation of *Armeria maritima* (Willd) ssp. *maritima* in the place "La Sărătură". Second issue is the ecology of that species and the relation between *A. maritima* (Willd) ssp. *maritima* and sodium chloride concentration in soil. Some studies mention that *A. maritima* (a few sspecies) can also uptake some heavy metals (Zn, Cu, Pb, Cd) through her pivoting roots (Baummach et Hellwig, 2003; Lukaszevska-Szarek et al., 2004; Neumann et al., 1995). Another issue that appears in this updated study refers to morpho-anatomical changes of *A. maritima* (Willd)'s stem. *A. maritima* (Willd) ssp. *maritima* that belongs to Plumbaginaceae Family, is a simple plant that presents flowers in capitulate inflorescences. The fruit is a monospermous capsule. The plant presents a hairy stem (Ciocârlan, 1990) but this aspect is problematic today. The pollen morphology is characteristic for Plumbaginaceae family. All the characteristics were investigated in our case.

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Materials and methods

All studies were developed during a period of six years. Most of the study consisted in field work. The second part of this study was developed in the Laboratories of Plant Physiology, Ecotoxicology and Morphoanatomy, from Faculty of Biology and Geology at Babeş-Bolyai Univesity, Cluj-Napoca, România. We used: microscope with photo camera, pH-meters, Ω-meters, some volumetric methods (for Cl^- quantitative determination). In all the determinations, repeatability was good.

Results and discussions

From morpho-anatomical point of view, *A. maritima* (Willd) ssp. *maritima* shows some characteristics that are very interesting. In the specialty literature, the plant stem is mentioned with a hairy surface (Ciocârlan, 1990; Lefèbvre, 1974) but *A. maritima* (Willd) ssp. *maritima* from 3 years ago had no stem with hairy surface. This aspect should be further studied. The pollen morphology hasn't change during this time and this aspect certifies that *A. maritima* (Willd) ssp. *maritima* has a stable genotype (Eisikowitch et Woodell, 1975; Lefèbvre, 1974). From physiological point of view, the soil concentration in NaCl is 2.85%, in NO_3^- is 0.177 % and copper concentration is 9.28 ppm. Some articles mention that *A. maritima* (Willd) ssp. *maritima* is a cuprophile plant, but in our case the quantity of copper in soil is too low to speak about *A. maritima* (Willd) ssp. *maritima* as being a cuprophile plant. With this dates, we can draw an overview regarding the ecophysiolgy of *A. maritima* (Willd) ssp. *maritima* from Blăjenii de Jos.

But the most important and interesting subject of this article is related to the phytosociology of *A. maritima* (Willd) ssp. *maritima*. In *Figure 1*, we observe that the number of individuals (*A. maritima* W. ssp. *maritima*) varies during the six years. The first population was monitored inside the fence and the second population was monitored outside the fence. The fence was an apparent protection for *A. maritima* W. ssp. *maritima* on the five hectares where the species lived in a large number.

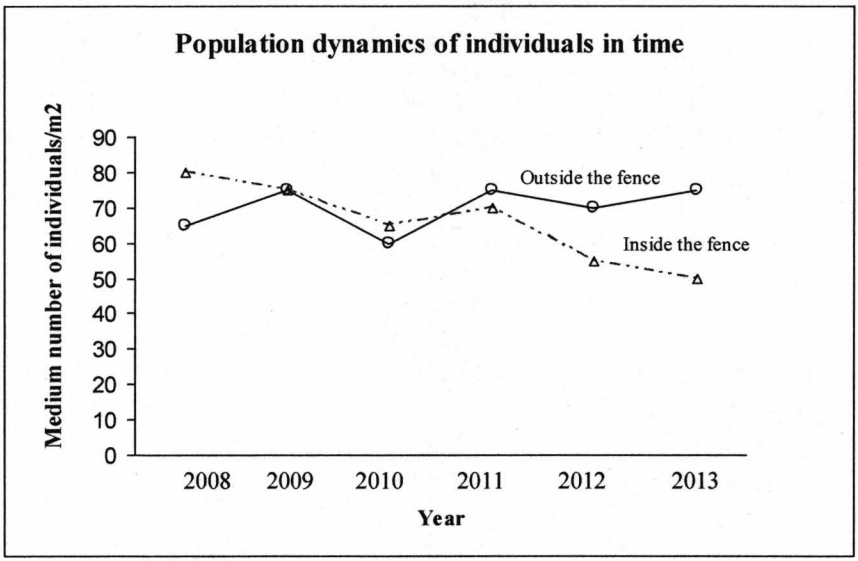

Fig. 1. Population dynamics of individuals in time

The situation in the second population is as follows: this population presents an increased of Medium number of individuals/m^2 because *A. maritima* W. ssp. *maritima* presents a vegetative breeding through fragmentation of root and rhizomes. Outside the fence, animals tread the roots and rhizomes of *A. maritima* W. ssp. *maritima* favoring vegetative reproduction. In laboratory, *A. maritima* W. ssp. *maritima* does not grow from seeds and in Nature reproduction through seeds is very rare (Köhl, 1997). On the inside of the fence, many weeds grow up smothering, in this situation, the development of *A. maritima* W. ssp. *maritima.* Those weeds were no longer grazed and *A. maritima* W. ssp. *maritima* was unable to grow. This situation demonstrate that the human action in natural areas is not so helpful most of the time and explains what increased the medium number of individuals outside the fence that delimited the 5 hectares under the name The Botanical Reservation "La Sărătură". This reservation proved not to actually protect *A. maritima* W. ssp. *maritima.*

The overview about ecology of *A. maritima* W. ssp. *maritima* in 2013 is an update in plant ecology, for Bistriţa-Năsăud county.

Rezumat

În localitatea Blăjenii de Jos, judeţul Bistriţa-Năsăud, în Rezervaţia Botanică "La Sărătură" habitează o plantă raportată ca fiind unică în România (Ciocârlan, 1990). Planta se numeşte *Armeria maritima* (Willd) ssp. *maritima* şi este o specie din Fam. Plumbaginaceae, specie de sărătură moderată. În prezentul studiu demonstrăm în principal acţiunea dăunătoare a omului în mediul natural. Iniţial, 5 ha din arealul speciei, pe care aceasta avea o densitate crescută au fost îngrădite cu scopul protejării

plantei de păşunatul animalelor agricole. În timp, lipsa acestor animale în interiorul gardului care delimita cele 5 ha, a dus la stingerea populaţiei de *A. maritima* W. din cauza faptului că ierburile, de regulă păşunate, au crescut în aşa măsură încât au înnăbuşit creşterea şi dezvoltarea speciei aparent ocrotite. În contrast, în exteriorul gardului, planta are la ora actuală o densitate medie mult mai mare decât cea prezentă la interiorul gardului. Un al doilea motiv cu care argumentăm acest fenomen este legat tot de păşunatul animalelor. Acestea călcau peste *A. maritima* W. şi îi fragmentau rizomul favorizând înmulţirea vegetativă a plantei. Lucrul acesta nu mai are loc în interiorul gardului însă este prezent la exterior din care cauză avem aceste evidente diferenţe în densitatea medie a speciei în cauză. Un ultim aspect interesant este faptul că specia nu se mai încadrează în descrierea din Flora ilustrată a României, ea nemaiavând scapul pubescent aşa cum este caracterizată în monumentala lucrare *Flora ilustrată a României*. Acest fenomen trebuie investigat mai amănunţit; noi intuim o legătură a prezenţei perilor pe scap, cu compoziţia solului şi cu factorii climatici. Din punct de vedere genetic planta este stabilă, morfologia polenului, de la an la an rămânând neschimbată cu toate că un caracter morfologic, iată, a dispărut.

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SYMBIOTIC NITROGEN-FIXING MICROORGANISMS - AN OVERVIEW

Rahela CARPA*

Abstract: Symbiotic nitrogen fixation in plants occurs in root nodules of legumes and nonlegumes. The bacterium *Rhizobium* is one of the most studied symbiotic nitrogen-fixing bacteria because it nodulates on legumes, which are environmentally significant in soil N fertility management of cultivated lands. The majority of nonlegume nodules belong to the *Alnus*-type symbiosis, in which the actinomycete *Frankia* is the microsymbiont. The cyanobacteria *Nostoc* and *Anabaena* nodulate the *Cycadales*, while the bacterium *Rhizobium* forms *Parasponia* type symbioses. This paper approaches the following subjects: symbiotic bacteria which produce radicular nodules at leguminous plants; symbiotic *Actinomycetes* which produce radicular nodules at non-legumes; symbiotic bacteria which produce foliar nodules; symbiotic *Cyanobacteria* which produce nodules and those which do not; nitrogen fixation by bacteria from associative symbioses.

Keywords: Symbiotic nitrogen fixation, *Rhizobium*, nodule.

Symbiotic bacteria which produce radicular nodules at leguminous plants

These bacteria belong to *Rhizobium* genus. *Rhizobium* species are found free in soil but they can fix nitrogen only in symbiosis with host plants in a controlled microaerophilic environment (Juarez et al., 2005).

Rhizobium–legume symbiotic interaction is an efficient model system for soil remediation and reclamation. Root nodule is a unique and highly organized structure developed as a result of the symbiotic relationship between leguminous plants and bacteria of the genus *Rhizobium*. This system has some advantages because of microorganism's ability to affect metals solubility, bioavailability, mobility and use of plants, legumes for phytoremediation. Rhizobium–legume symbiosis is an efficient system for soil nitrogen improvement (Mandal et al., 2011).

The group of rhizobia is considered one of the most powerful P-solubilizers and some of them, such as *Rhizobium leguminosarum* are able to mobilize phosphorous to plants (Rodriguez and Fraga, 1999; Abril et al., 2003). Species from genus *Phaseolus*, indigenous from American continent, are one of the most important legumes for human nutrition. Currently, six rhizobial species have been identified in common bean nodules (Velazquez et al., 2001).

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Symbiotic rhizobia are common colonizers of the rhizosphere of both legume and nonlegume plants (Table 1) and in addition to legumes they are also endophytes of several nonlegumes like rice and maize (Sessitsch et al., 2002). However, nonsymbiotic rhizobia can also be present in soil (Sullivan et al., 1996). Rhizobia can be isolated either by collecting nodules from field grown legumes or by inducing nodule formation by inoculating surface disinfected legume seeds with soil suspensions under aseptic laboratory conditions.

Table 1. Species or root and stem-nodulating bacteria and their hosts

Rhizobial species:	**Host legumes:**
Rhizobium meliloti	*Medicago, Melilotus, Trigonella*
Rhizobium fredii, R. xinjiangensis	*Glycine max, G. soja, and other legumes*
Rhizobium leguminosarum *bv. viciae* *bv. trifolii* *bv. phaseoli*	 *Pisum, Vicia, Lathyrus spp, Lens culinaris* *Trifolium spp.* *Phaseolus vulgaris*
Rhizobium tropici	*Phaseolus vulgaris, Leucaena spp.*
Rhizobium etli	*Phaseolus vulgaris*
Rhizobium galegae	*Galega officinalis, G. orientalis*
Rhizobium loti	*Lotus spp.*
Rhizobium huakuii	*Astralagus sinicus*
Bradyrhizobium japonicum, B. elkanii, B. liaoningense	*Glycine max*
Sinorhizobium meliloti, S. medicae	*Medicago spp., Melilotus spp.*
Sinorhizobium fredii, S. xinjiangense	*Glycine max*
Mesorhizobium loti	*Lotus corniculatus*
Mesorhizobium ciceri *Mesorhizobium mediterraneum*	*Cicer arietinum*
Mesorhizobium huakuii, M. septentrionale, M. temperatum	*Astragalus cicer, Astragalus sinicus, Astragalus adsurgens*
Azorhizobium caulinodans	*Sesbania rostrata*

Source after: Olsen et al., 1996; Sahgal and Johri, 2003; http:== www.rhizobia.co.nz =Rhizobia_Taxonomy.html.

In the initial phase the roots excrete some substances with positive chemotactic effect on the specific rhizobium of the respective plant. These substances are lecithines (glycoproteins) which recognize the common antigenic sites, present both on the surface of root hairs and on the specific rhizobium and, thus, as a biochemical bridge, tie the specific rhizobium cells to the root hairs. Then rhizobia penetrate the root hairs. After rhizobia penetration, the hairs are distorting (their tip becomes bent) (Figure 1). It is supposed that

the deformation is owed to β-indolylacetic acid produced by rhizobia. Not all the infected hair roots produce nodules afterwards (usually only 5%).

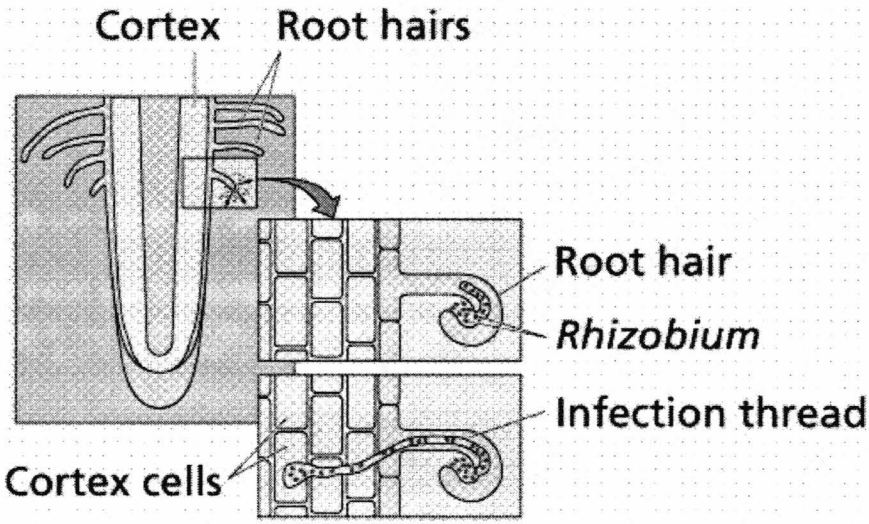

Fig. 1. Infection thread in root hairs
(http://www2.estrellamountain.edu/faculty/farabee/biobk/biobookplanthorm.html)

In the hair roots an infection thread forms, penetrating the radicular cortex (Figure 2). The infection thread is built-up by chains of rhizobia cells and by a celluloso-pectic wall which surrounds the rhizobia chains. The thread wall constitutes the prolongation of the wall of radicular cells and is developed by the wall as a reaction at rhizobia invasion (Probanza et al., 1996).

The infection thread branches, than the rhizobia within it are freed and arrive in the cytoplasm of the radicular cells. Simultaneous with the release of rhizobia from the infection thread the host cells multiply swiftly and in their cytoplasm the rhyzobia multiply. Thus the nodule is formed, wherein the rhizobia evolve towards the stage of bacteroids (Figure 2).

In the final structure of the nodule a central zone and a peripheral zone differentiate. In the central zone the cytoplasm of the tetraploid cells contains plural aggregates of 4-6 bacteroides tunicated by a lipoprotein membrane of plant origin and surrounded by a protein rich cytosol, which includes leghemoglobin. The peripheral zone is formed of diploid cells lacking bacteroides, this zone contains the vascular system of the nodule, whose formation depends on the presence of plant available boron (Drăgan-Bularda and Kiss, 1986).

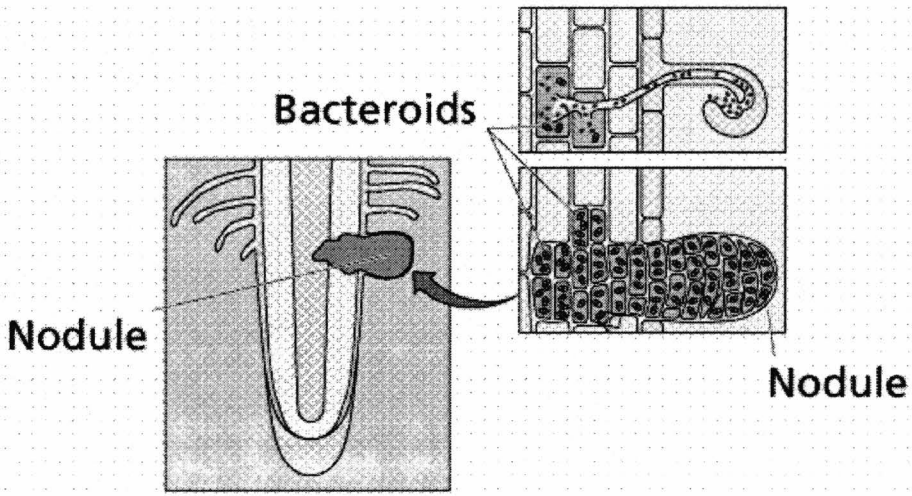

Fig. 2. Bacteroid stage
(http://www2.estrellamountain.edu/faculty/farabee/biobk/biobookplanthorm.html)

Various researches demonstrated the ability of *Rhizobium* to colonize roots of non-legumes (Matiru and Dakora, 2004) and act as phytohormone producer, phosphate solubilizer and to some extent as nitrogen fixer (Afzal and Bano, 2008).

Various phenotypic and genotypic methodologies are being used to identify and characterize bacteria. Although phenotypic methods play a significant role in identification but the molecular methods are more reliable and authenticated for identification and to study genetic diversity of bacterial isolates. Major molecular techniques include PCR (Polymerase chain reaction), RAPD (randomly amplified polymorphic DNA), RFLP (restriction fragment length polymorphism), AFLP (amplified fragment length polymorphism), SSR (single sequence repeats) and 16S-rRNA gene sequencing (Naz et al., 2009).

For isolation of *Rhizobium* species 10 g rhizosphere soil were suspended in 90 ml of sterile distilled water. Serial dilutions (10x) were made with 9 ml distilled water and an aliquot (100 µl) from decimal dilutions was used to inoculate YEMA (Vincent, 1970) media for the growth of *Rhizobium*. After incubation time, to measure the survival efficiency, colonies of *Rhizobium* from 10^8 dilutions were counted following the formula adapted by James (1978):

$$Viable\ cell\ count\ (CFU\ /\ g\ soil) = \frac{Number\ of\ colonies}{Volume\ of\ inoculum} \times Dilution\ factor$$

Based on the morphologic appearance of the nodules the effectiveness of nitrogen fixation can be assessed (the capacity of nodule rhizobia to fix N_2). If the nodules are numerous and small it means that rhizobia are less effective.

Sparse, big and red nodules denote effective rhizobia. The red colour is due to leghemoglobin, which forms only in the nodules of legumes (the plant alone or the rhizobia alone can not synthetize leghemoglobin. It was experimentally proved that between the leghemoglobin content and the effectiveness of N_2 fixation there is a significant correlation, so it participate in the N_2 fixation mechanism in the nodules.

In order to increase the crops of legumes, their seeds are treated, before sowing, with very effective rhizobia strains (seeds bacterisation). The inoculum containing rhizobia for bacterisation of seeds are obtained on industrial scale. These „bacterial fertilizers" are commercialized under different names, in our country as nitragin (Drăgan-Bularda and Kiss, 1986).

Symbiotic Actinomycetes which produce radicular nodules at non-legumes

Radicular nodules from alder species (*Alnus*) contain an actinomycete (*Frankia alni*). If this lacks the alder seedlings do not develop. The N_2 fixing was proven by $^{15}N_2$ method and by acetylene reduction method: the nodules removed from the roots fix small amounts of $^{15}N_2$ and produce ethylene from acetylene, in the first hours after removal (Sellstedt and Lindlab, 1990).

The radiculare nodules were also depicted at other trees and shrubs from different genera (*Elaeagnus, Hippophae, Ceanothus, Coriaria, Myrica, Casuarina*, etc.). These nodules also contain actinomycetes which, based on host distinctiveness, were placed into 9 species of *Frankia* (Callaham et al., 1979).

There are two types of nodules. At some plants (*Alnus, Elaeagnus, Hippophae*, *Ceanothus, Coriaria* etc.), the nodules are coralloid. These are formed by continuous ramification of the side root deformed under the action of the actinomycete (Huguet et al., 2001). The second type is called rhizothamnia and comprises nodules covered with radicules which grow upwards (geotropic negative radicules). Rhizothamnia are found at plants from genera *Myrica*, *Casuarina* (Dawson et al., 1989).

Symbiotic bacteria which produce foliar nodules

At some tropical plants (*Psychotria, Pavetta, Ardisia, Grumilea*) foliar nodules are formed. At *Psychotria* species it was proved that the nodules contain symbiotic bacteria of *Klebsiella* genus and fix N_2 (Mihăescu and Gavrilă, 1989).

Symbiotic Cyanobacteria which produce nodules

Nitrogen fixing, free cyanobacteria, from *Anabaena* and *Nostoc* genera, can be symbiotic, producing radicular nodules on some gymnosperms (*Cycas, Macrozamia, Encephalartos* etc.). These nodules fix N_2.

Symbiotic Cyanobacteria which do not produce nodules

Cyanobacteria of *Anabaena* and *Nostoc* genera live in symbiosis also with fungi (forming lichens), liverworts, ferns and angiosperms. Thus, the lichens from *Peltigera* and *Collema* genera contain N_2 fixing *Nostoc*. At liverworts *Anthoceros laevis* and *Blasia pusilla* the symbiont is *Nostoc lichenoides*. *Anabaena azollae* lives and fixes nitrogen in leafs of the aquatic pteridophyte *Azolla*. Among angiosperms, *Gunnera* species live in symbiosis with N_2 fixing cyanobacteria, which develop in the glands place at the base of leafs (Brock, 2000). Unicellular filamentous cyanobacteria fix nitrogen at dark. *Synechocystis sp.* BO8402 is characterized by an increased autofluorescence level originating in the paracrystalline phycobiliprotein complexes located in the cell inclusions. The transfer of electrons between paracrystalline phycobiliproteins and photosystems is ineffectual. The formation of paracrystalline phycobiliproteins in *Synechocystis sp.* BO8402 represents a mean unknown for unicellular cyanobacteria to fix nitrogen at light (So et al., 2002).

Nitrogen fixation by bacteria from associative symbioses

The nitrogen fixing microorganisms which live freely, frequently associate with the plant roots or leafs, wherein nutritive substances are found, especially carbohydrates at which they multiply and fix nitrogen, resulting mutual beneficial associations. Such associations between free nitrogen fixing organisms and the plant roots are called rhizocoenosis and the zone where it occurs is called rhizosphere. The rhizosphere is the soil zone where the direct influence of the radicular system is felt, which causes microorganisms proliferation, adding the foliar exudates washed by precipitation and the decomposed fallen leaves (Klassen et al., 2005).

Many bacteria participating in symbiosis relationships were described. *Azotobacter paspali* was found in the rhizosphere of some varieties of the tropical gramineous plant *Paspalum notatum*. Other *Azotobacter* species were found in the rhizosphere of the barley and other plants. *Beijerinckia indica* was found in the rhizosphere of sugar cane, rice and other plants. *Azospirillum lipoferum* and *Azospirillum brasilense* live in the rhizosphere of most tropical and subtropical gramineous fodder plants, of maize and rice corn, also producing auxines needed in plant growth (Bloemberg and Lugtenberg, 2001). From the rhizosphere of different plants were detected other nitrogen fixing bacteria like *Derxia, Bacillus, Klebsiella, Clostridium, Desulfovibrio, Enterobacter* (Rönkkö et al., 1993).

Bacteria from associative symbioses are ectorhizospheric, living in the soil surrounding the roots and on the root surfaces. *Azospirillum* species can also be endorhizospheric, living either intercellularly in the root tissues or inside the cortical cells (Rutkowski et al., 2006).

Conclusions

In this paper a few aspects regarding nitrogen fixing microorganisms were described. There are symbiotic microorganisms that produce radicular or foliar nodules in symbiosis with different groups of plants.

Rezumat

Fixarea simbiotică a azotului la plante are loc în nodozităţi radiculare prezente la leguminoase, dar şi la non-leguminoase. *Rhizobium* este unul dintre cele mai studiate grupuri de bacterii fixatoare de azot simbiotice, deoarece produce nodozităţi la leguminoase, care au importanţă deosebită legată de fertilitatea solului şi de azotul disponibil în solurile cultivate. Majoritatea nodozităţilor produse la nonleguminoase aparţin simbiozei cu specii de *Alnus*, în care actinomicetul *Frankia* este microsimbiontul. Cianobacteriile *Nostoc* şi *Anabaena* formează nodozităţi la ordinul *Cycadales*, în timp ce rizobiile formează simbioze cu *Parasponia*. Această lucrare este legată de următoarele subiecte: bacterii simbiotice care produc nodozităţi radiculare la plantele leguminoase; *Actinomycetes* simbiotice care produc nodozităţi radiculare la plantele non-leguminoase; bacterii simbiotice care produc nodozităţi foliare; *Cyanobacteria* simbiotice care produc sau nu produc nodozităţi; bacterii fixatoare de azot din simbiozele asociative.

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CELL SIGNALLING BY MAPK FAMILY - MINIREVIEW (MAPK - *mitogen-activated protein kinase*)

Andreea CRINTEA*

Abstract: Protein kinases are important elements for controlling intracellular processes, about 3% of all the eukaryotic functional genes codifying protein kinases. Initially, protein kinases were identified at yeasts, but genes codifying protein kinases were also discovered at plants (Heldt and Piechulla, 2005). The superfamily of eukaryotic protein kinases was divided into four large kinase groups, all being closely related with each other (Storey, 2004). The pathways involving MAPK are the most important signalling pathways discovered at eukaryotes and probably the most conserved ones (Perotto and Baluška, 2012).

By using numerous plants, members of MAPK, MAPKK and MAPKKK families could be identified (Bögre and Beemster, 2010; Heldt and Piechulla, 2005).

At plants, the cell signalling induced by MAPK includes a cascade reaction of MAPK type, which operates in series: MAPKKK, MAPKK and MAPK (Yang, 2008).

Keywords: protein kinase, *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, *Arabidopsis*, MAPK.

Introduction

Protein kinases and protein phosphatases are important for regulating the intercellular processes. It is estimated that 1 to 3 percent of functional genes in the eukaryotic cell codify protein kinases (Heldt and Piechulla, 2005). In eukaryotes two large subgroups of protein kinases are found, Ser/Thr kinases (serine/threonine kinases) and Tyr kinases (tyrosine kinases). Initially, protein kinases were identified at yeasts (Heldt and Piechulla, 2005). It was emphasised that *Saccharomyces cerevisiae* genome codifies 114 Ser/Thr protein kinases (and not Tyr kinases) (Storey, 2004). Afterwards, genes codifying protein kinases were also discovered at plants (Heldt and Piechulla, 2005) and, by sequencing the *Caenorhabditis elegans* genome, the existence of 92 Tyr kinases was proven (Storey, 2004).

The subfamily of eukaryotic protein kinases was divided into four large groups of kinases: an AGC group, consisting of PKA and PKG (Cyclic nucleotide-regulated family), PKC (calcium/phospholipid-dependent family); CaMK group (calmodulin-dependent kinase group); CMGC group (cyclin, mitogen, glycogen, casein kinases) and PTK group. Each of these groups has a close connection with the others, these proteins being homologous and

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descending from a common ancestor. There is quite a large number of kinases which do not fall into one of these major groups, for instance histidine kinases and aspartate/glutamate kinases (Storey, 2004). In fungi, animals or plants, most protein kinases contain 12 conserved regions and can phosphorylate the –OH group from serine and/or threonine and in some cases even tyrosine (Heldt and Piechulla, 2005).

1. MAPK (mitogen-activated protein kinase)

Mitogen – term for a multitude of substances, many of them of unknown nature. These substances can be involved in the stimulation of mitosis but also in other reactions. For instance, G proteins and also phytohormones may act as mitogen factors (Heldtand Piechulla, 2005).

The medium molecular mass of MAPK (mitogen-activated protein kinase) is between 42 and 53 kDa. By crystallography, it was proven that all the members of MAPK family present similar 3D structures and the functions are conserved (Taj et al., 2010).

MAPK plays an important role in the protein kinase cascades and regulation can take place by phosphorylation with the aid of other protein. For instance, in a such cascade reaction, the G protein activates MAPKKK (MAP kinase kinase kinase), it activates by phosphorylation MAPKK (MAP kinase kinase), in turn it activates MAPK (MAP kinase), than it phosphorylates different substances. By phosphorylating multiple transcription factors MAPK has an important regulating function in processes of differentiation and cell development, thus regulating expression of different genes (Heldt and Piechulla, 2005).

The pathways involving MAPK are presumably the most conserved and the most important signalling pathways discovered in eukaryotes (Perotto and Baluška, 2012).

2. Cell signalling by MAPK family in plants

By using multiple model plants, the members of MAPK, MAPKK and MAPKKK families could be identified. In the *Arabidopsis* genome 20 MAPK, 10 MAPKK and 60 MAPKKK were found (Heldt and Piechulla, 2005). The *Oryza sativa* and *Populus trichocarpa* genomes were sequenced and it was noticed that codify 15, 21 MAPK and 8, 11 MAPKK, respectively. Unlike human or yeasts, plants have more MAPK and MAPKK. More than 60 genes from *Arabidopsis* genome codify MAPKKK, but not all of these kinases function as MAPKKK, one of these being MAP3Kε1 (Bögre and Beemster, 2010). Ethylene is one of five classic hormones found in plants, being discovered at the beginning of 20th century (Hahn and Harter, 2009). The effect caused by ethylene results from the alteration of gene expression. This classic hormone, ethylene, same as other phytohormones, carry out his effect at low concentrations (Heldt and Piechulla, 2005).

Cell signalling induced by ethylene includes a cascade reaction of MAPK type, which regulates many aspects of growth and development in plants, including cytokinesis. This cascade picks up stimuli from different membrane receptors and conducts them to intracellular targets. Thus, the perpetuation of a short lasting stimulus is provided, at receptor level, and his amplification throughout the cell. This action is facilitated by the existence of a large number of MAPK proteins, which belong to three distinct classes, which operate serially: MAPKKK, MAPKK and MAPK (figure 1).

About the ethylene receptor in plants, the first one identified, by means of mutational studies at *Arabidopsis*, was ETR1 (ethylene response 1), the mutant for this protein being insensitive to exogenous application of ethylene. The identification of the DNA which codifies the protein shown a strong similarity with the cell signalling system composed of two components, of histidine kinase type (Jones, 2012).

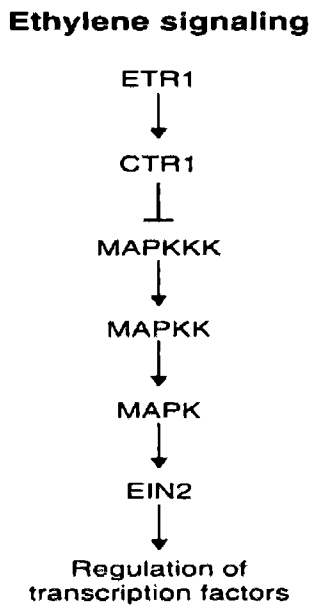

Fig. 1. The cell signalling induced by ethylene includes a cascade of MAPK (Jones, 2012)

ETR1, similar with the other receptors identified later, has a transmembrane domain at the N-terminal end, which binds ethylene, and a histidine kinase one at the C-terminal end. The ethylene receptor needs the presence of a copper atom in order to function. The receptor can be active in the presence of ethylene, thus inhibiting the response pathway, and in the presence of ethylene the receptor is inhibited, the pathway is unlocked and starts the transcription of prior suppressed genes. The MAPK cascade functions downstream ETR1

through a second protein, equally with kinasic activity, which inhibits the genes for ethylene. The actual way in which the signal is conducted from CTR1 (Ser/Thr kinase), by MAPK cascade, to EIN2 (ethylene insensitive-2), another protein with transmembrane domains, and further to the nuclear transcription factors EIN3 and ERF1 (ethylene response factor-1) is not known (Hedden and Thomas, 2006, Yang, 2008). The function of EIN2 protein remains unknown. The geneticists place this membrane protein in the signalling pathway induced by ethylene between CTR1 and EIN3, but the mechanism that connects it with the components of the upstream pathway and with the EIN3 nuclear transcription factors, located downstream, is not known (Hahn and Harter, 2009).

Conclusions

Protein kinases, first, have been identified in yeast, and also in plants.

Eukaryotic protein kinases superfamily is divided in four groups of kinases. The pathways in which is involved MAPK are the most important pathways that have been discovered at eukaryotic organisms and are probably the most preserved.

Using a large number of plants, they could identify the members of MAPK, MAPKK and MAPKKK families. In plants, the pathway which is induced by MAPK, includes cascade reactions which operates in series: MAPKKK, MAPKK and MAPK.

Rezumat

Protein kinazele sunt elemente importante pentru reglarea proceselor intracelulare, aproximativ 3% din totalul genelor funcţionale eucariote codificând proteine kinaze. Iniţial protein kinazele au fost identificate la drojdii, însă au fost descoperite şi gene ce codifică protein kinaze şi la plante (Heldt şi Piechulla, 2005). Superfamilia protein kinazelor eucariote a fost împărţită în patru mari grupuri de kinaze, fiecare dintre acestea fiind în strânsă legătură unele cu celalte (Storey, 2004). Căile în care este implicată MAPK sunt cele mai importante căi de semnalizare care au fost descoperite la eucariote şi probabil cele mai conservate căi (Perotto şi Baluška, 2012).

Utilizând numeroase plante, s-au putut identifica membrii ai familiilor MAPK, MAPKK şi MAPKKK (Bögre şi Beemster, 2010, Heldt şi Piechulla, 2005).

La plante, semnalizarea celulară indusă de MAPK include o cascadă de reacţii de tip MAPK, MAPK ce operează în serie: MAPKKK, MAPKK şi MAPK (Yang, 2008).

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ATTRACTING THE USEFUL BIRDS IN PARKS AND GARDENS USING ARTIFICIAL NESTS

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Claudiu GAVRILOAIE***

Abstract: The usage of artificial nests significantly contributes to the improvement of our knowledge concerning the ecology, behaviour and phisiology of the insectivorous birds nesting in hollows. Thus we can make a monitoring of the eggs laying, offsprings emerging from eggs and their development until they leave the nest. The material, the shape and the orientation of the artificial nests have a major importance for the experiment. In this paper we used 30 artificial nests which were placed in the Park of the Regional Centre of Francophony, Arcalia, Bistrița-Năsăud county. The nest were observed in the spring season of 2012.

Keywords: artificial nests, insectivorous birds, biological combating, eggs and offsprings monitoring.

Introduction

Having in mind the importance of insectivorous birds in the maintenance of dynamic equilibrium and the stability of the biocenosys, we intended to attract these birds in order to fight against the harmful using artificial nests (Ardia et al., 2006).

The researches concerning the quantitative and qualitative food composition of the birds have shown that the most insectivorous birds from forests, orchards ans open habitats eat eggs, larvae, caterpillars, case worms and harmful insects in a significant amount (Kórodi,1972).

When we evaluate the role of the insectivorous birds in combating harmful insects, we have to consider the following aspects: the frequency they consume harmful insects, the ratio between the total amount of food intake and the harmful insects amount, the infleunce of birds upon the harmful insects populations, the importance of birds protection in combating harmful insects and the possibilities to combine these actions with other biological combating measures (Berkesy and Berkesy, 1999).

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The study place

The study was made in spring of 2012, at the Regional Centre of Francophony, Arcalia, Bistriţa-Năsăud county (Fig. 1). The park is situated on the left side of river Şieu, at the west side of Arcalia village. it has a surface of 18 hectares (670 m lenght and 350 m width). The park has good conditions for useful birds reproduction because in park there are many natural hollows, usually there is a water source, natural enemies are rare and the introduction of artificial nests increases the birds density.

Fig. 1. Regional Centre of Francophony, Arcalia

In orchards, the attraction of useful birds (especially the tits - Paridae) has a major importance since they consume many harmful insects of the fruit trees. Even we can not determine these birds (Paridae) to establish in orchards for nesting, we can feed them supplementary in the winter season, thus making them to come more frequent in orchards.

The park vegetation is very diverse. The trees were planted in an old meander, thus avoiding the cold air currents. There are indigenous and exotic deciduous and resinous trees of 30-40 m height (Szabó and Zăpîrţan, 1965).

Beside trees there are different shrubs forming from place to place hard penetrable thickets. The natural herbaceous vegetation forms xerofilous and mezo-xerofilous meadows (Rösler, 1965).

This great vegetable diversity is favored by the increased air humidity through the entire year. the abundant precipitations infiltrate in the soil, fact which leads to the abduction of freatic layer (Osváth and Gălan, 1972).

The vegetation provides best feeding and nesting conditions for a great variety of birds living in the park (Vertse, 1975).

Method

We have made 30 artificial nests. Thew were made from OBS wood of 15 mm thickness. We have chosen the nests of type B, with sides of 20x12 cm, and the entrance hole of 32-35 mm diameter, at 15 cm height from the nest bottom, with a mobile hood (Fig. 2-3). The nests were placed on different trees at 3-6 m height, exposed to different cardinal points (Fig. 4). The nest were sistematically controlled during the entire nesting season.

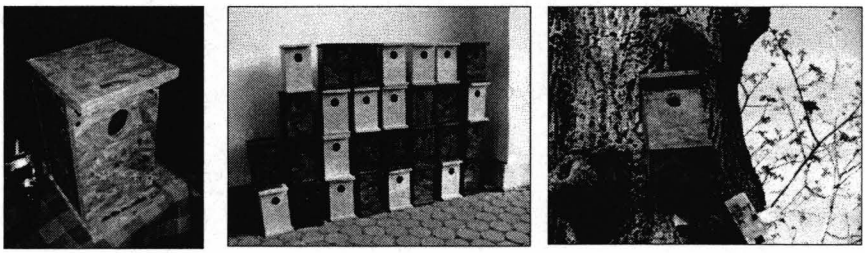

Fig. 2. Artificial nests of type B

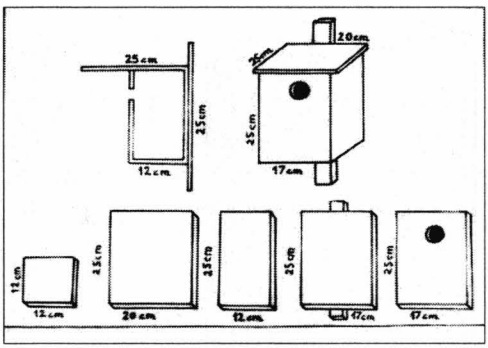

Fig. 3. The scheme of type B nest

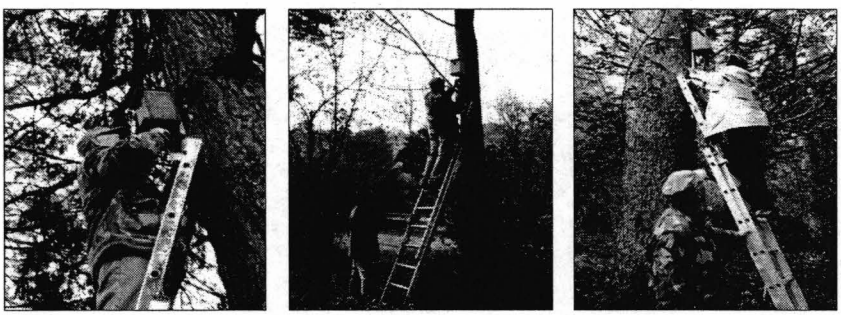

Fig. 4. Artificial nests assembling

Results and discussions

The number of occupied nests, bird species, number of eggs and the offsprings raised in the nests during the study period are presented in Table 1.

Six nests were occupied by the nuthatch (*Sitta europaea* Wolf.). This species nests between April and June. The number of the layed eggs was between 7 and 8 each nest (Fig. 5). The nest is coated with dried leafage and tree bark fragments. The nesting period lasts 13 days. During the first eggs laying, 39 eggs were layed in the 6 nests; 34 offsprings were born, 2 died and 3 eggs were unfertile (Table 1).

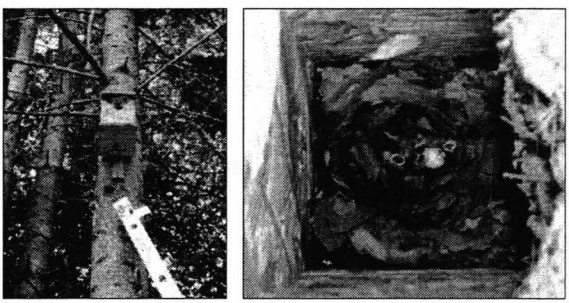

Fig. 5. Nests, eggs and offsprings of *Sitta europaea* Wolf

Three nests were occupied by Eurasian tree sparrow (*Passer montanus* L.). The species nests between April and August. The nesting period lasts 13-14 days. The number of the layed eggs was 5-6 each nest. The nests were coated with grass, straw and feathers. There were 18 eggs from the three nests, of which 2 were unfertile, 13 offsprings emerged and other three died (Table 1).

Eight nests were occupied by great tit (*Parus major* L.). This species nests between April and July and it lays 8-12 eggs. The nesting period lasts 13-15 days and the offsprings development last another 17-18 days. The nests were coated with moss and bristle. There were 67 layed eggs in the eight nests; from which 4 egges were unfertile, 5 offsprings died and other 58 remained (Fig. 6 & Table 1).

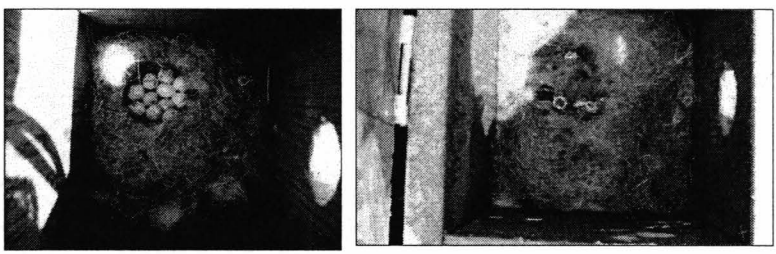

Fig. 6. Eggs and offsprings of *Parus major* L.

Six nests were occupied by blue tit (*Parus caeruleus* L.). It nests between April and July, for 13-15 days and the offsprings development last another 17-18 days. The nests were coated with moss and bristle. Forty-one eggs were

layed, from which 6 were unfertile, 4 offsprings died and other 31 remained (Table 1).

Table 1. Bird species, occupied and unoccupied nests, and offsprings number

No.	Species name	Occupied nests		Layed eggs and grown offsprings		Unfertile eggs and dead offsprings	
		number	%	eggs	offsprings	eggs	offsprings
1	Nuthatch *Sitta europaea* Wolf.	6	20	39	34	3	2
2	Eurasian tree sparrow *Passer montanus* L.	3	10	18	13	2	3
3	Great tit *Parus major* L.	8	26	67	58	4	5
4	Blue tit *Parus caeruleus* L.	6	20	41	31	6	4
5	Unoccupied nests	7	24	-	-	-	-
	Total 30	30	100	165	136	15	14

During the spring season of 2012, in our artificial nests 4 bird species have nested. They occuoied 76% of the nests. the 4 species raised 136 offsprings (4.5 being the average for each nest). So, were 1.3 bird pairs on each hectare, besides the natural nests. Such birds density is very useful in combating a great number of harmful insects.

Conclusions

The majority of artificial nests were occupied by small birds as nuthatch, Eurasian tree sparrow, great tit and blue tit. There were some unoccupied nests (24%). This happened not because the nests dimensions or orientation but the long and cold winter, fact which probably produced the death of a great number of birds.

Using artificial nests is a good opportunity to attract useful birds in different habitats. These birds can significantly contribute to the harmful insects combating, thus avoiding the negative efects of chemical substances and harmfuls invasions.

We propose the introduction of 5-6 artificial nests on each hectare, especially in the habitats which are exposed to harmfuls attacks.

Rezumat

Utilizarea cuiburilor artificiale contribuie semnificativ la îmbunătăţirea cunoştinţelor noastre în ceea ce priveşte ecologia, comportamentul şi fiziologia păsărilor care cuibăresc în scorburi, în special insectivorele. Utilizând metoda cuiburilor artificiale se poate realiza monitorizarea depunerii ouălor, a ecloziunii puilor şi dezvoltarea acestora până la părăsirea cuiburilor. Materialul, forma şi orientarea cuibului artificial au o importanţă deosebită în realizarea experimentului. În lucrarea de

faţă am utilizat 30 de cuiburi artificiale pe care le-am montat în Parcul Centrului Regional al Francofoniei Arcalia, jud. Bistriţa-Năsăud şi pe care le-am monitorizat în primăvara anului 2012.

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THE DYNAMICS OF THE CHAMOIS (*RUPICAPRA RUPICAPRA CARPATHICA*) IN RODNA MOUNTAINS NATIONAL PARK (BIOSPHERE RESERVE)

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Abstract: The chamois (*Rupicapra rupicapra carpathica*) is a flagship species for Rodna Mountains National Park (Biosphere Reserve). The study was done having in mind the surface of 47.000 ha. The dynamic of chamois during 35 years is showing oscillations regarding the number of individuals because of human impact (poaching), disease. Nowadays the population is stable and the number is increasing.

Keywords: chamois, assessment, flagship.

Description of the area

Rodna Mountains National Park is a protected areas situated in Eastern Carpathians, northern part of Romania, near the border with Ukraine. Has a surface of 47.000 ha, was established in 1990 but the first protected nucleus was declared in 1932 (Pietrosu Mare). Later, in 1980, Pietrosu Mare was included in the UNESCO patrimony, being declared as a Biosphere Reserve (Bereş, 1989). The study area for chamois is represented by the surface of national park. The protected areas are comprising a high diversity of ecosystems: rocks, pastures, grasslands, mountain forests, subalpine shrubs, meadows, bogs, glacial circuses, glacial lakes, more than 20 peaks over 2.000 m altitude (Nădişan, 2000).

Rodna Mountains boost the highest peaks of Eastern Carpathians (Pietrosu Mare, 2.303 m) and they are placed in north of Romania (www.parcrodna.ro). They belong to the Northern Carpathian group, known as Maramureş and Bucovina Carpathians, and dominate the boundary region, the highest altitude gap being recorded towards Maramureş Depression, which is placed in north of Romania, Eastern Carpathians, counties of Maramureş, Bistriţa-Năsăud and Suceava (Coldea, 1990). Glacial landscape is well developed on the northern slope where some glacial circuses can be found (Pietrosu, Buhăescu, Negoescu etc.). On the southern slope, the glacial landscape is less impressive: some suspended glacial circuses and snow niches.

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Materials and methods

The chamois (*Rupicapra rupicapra*) is a goat species native to mountains in Europe, including the Carpathian Mountains of Romania, the European Alps, the Tatra Mountains, the Balkans, parts of Turkey, and the Caucasus. In Romania is present a subspecies *carpathica* (www.wikipedia.org).

Female chamois and their young live in herds of up to 100 individuals; adult males tend to live solitarily for most of the year. During the rut (late October/early November in Europe), males engage in fierce battles for the attention of unmated females. An impregnated female undergoes a gestation period of 170 days, after which a single kid is born in May or early June, although rarely twins may be born. If a mother is killed, other females in the herd may try to raise them (Couturier, 1938). The kid is weaned at 6 months of age and is fully grown by 1 year of age. However, the kids do not reach sexual maturity until they are 3 to 4 years old, although some females may mate at as early 2 years old. At sexual maturity, young males are forced out of their mother's herds by dominant males (who sometimes kill them), and then wander somewhat nomadically until they can establish themselves as mature breeding specimens at 8 to 9 years of age (Macdonald, 1993).

Chamois eat various types of vegetation, including highland grasses and herbs during the summer and conifers, barks and needles from trees in winter. Primarily diurnal in activity, they often rest around mid-day and may actively forage during moonlit nights. Chamois can reach an age of 22 years in captivity, although the maximum recorded in the wild is from 15 to 17 years of age. Common causes of mortality can include pouching, avalanches, epidemics and predation. The main predators of chamois are Eurasian lynxes and gray wolves, although a few may predate by brown bears and golden eagles as well. The main predator of chamois now is humans. Chamois usually use speed and stealthy evasion to escape predators and can run at 50 kilometers per hour and can jump 2 m vertically into the air or over a distance of 6 m (Macdonald, 1993).

The Park Administration has chosen the chamois as a target species for monitoring. The assessment of chamois population in Rodna Mountains becomes a priority for the Park Administration since 2004 because this species was threatened to extinction because of poaching. Since 2004, the administration of protected area is organizing twice per year a huge campaign of assessment (May-June and October-November - breeding period) with local NGOs, museums, researchers, forest engineers from forestry districts, volunteers, mass-media, students, other custodians for collecting real information about the effective population, distribution, sex ratio, threats. This information is very helpful for identifying the trend of chamois in the national park. The data took into consideration for identifying the dynamic of chamois was also used from forestry districts before the designation of Park Administration, thus mean assessments in the period 1977-2003, and after that

(2004-2013) new data for spring and autumn from the campaigns organized by the Park Administration.

The information collected using the biannual assessment of a target species - *Rupicapra rupicapra carpathica* (2004-2013) was introduced in a database. According to this database, can be drawn clear conclusions about the dynamic of chamois in Rodna Mountains and can be taken specific management measures for conservation the species, maintaining and/or strengthening the conservation status.

The principle behind this method of assessment is that of direct observation in the field, that in each ex-hunting paths which are overlapping on the surface of the park, the team of observers are verifying using binoculars and counting each individual of chamois for 18 trails. All individuals seen are mentioned in the form and centralized at the park administration. More than 100 volunteers are participating in each chamois assessment.

Results and discussions

The number of individuals counted in the period 1977-2013 is presented in the table 1 and depicted in the figure 1. In the period 1977-2003, the assessment was organized once per year and in the period 2004-2013 twice per year. We take into account for the period 2004-2013, the assessment from autumn.

Table 1. Assessment of chamois in Rodna Mountains in the period 1977-2013

No	Year of assessment	Number of chamois
1	1977	88
2	1978	115
3	1979	155
4	1980	170
5	1981	185
6	1982	303
7	1983	220
8	1984	350
9	1986	260
10	1987	285
11	1988	300
12	1989	295
13	1990	323
14	1991	345
15	1992	330
16	1993	335
17	1995	330
18	1996	280
19	1997	190

No	Year of assessment	Number of chamois
20	1998	140
21	1999	120
22	2000	115
23	2001	115
24	2002	113
25	2003	92
26	2004	61
27	2005	77
28	2006	109
29	2007	77
30	2008	140
31	2009	37
32	2010	126
33	2011	128
34	2012	159
35	2013	112

The maximum number of chamois was recorded in 1994 when the management of area was well maintained by the Forestry District from Borsa (Maramureş). Also, the assessment was recorder also outside of protected area. After 1995, the population of chamois was decreasing because of non management of the area until 2004 when the Park Administration was designated and since than, the population is increasing. In 2009 was counted a low number of chamois.

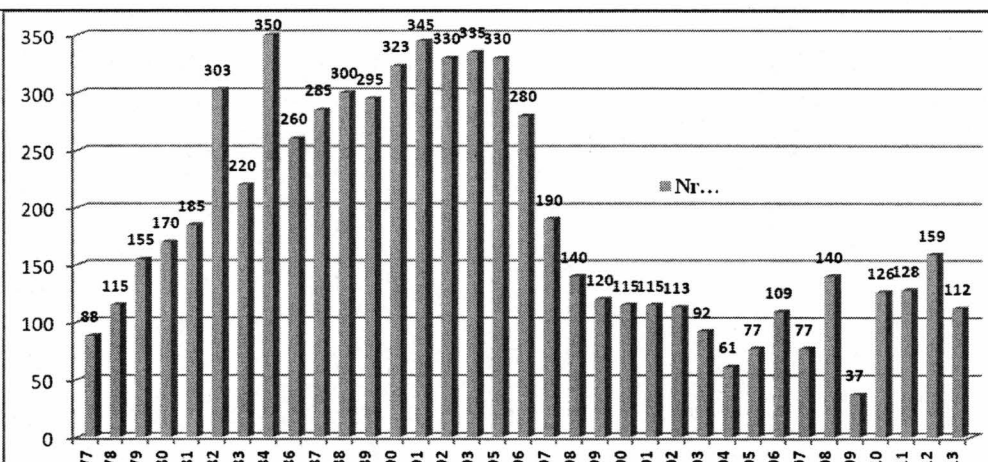

Fig. 1. The dynamic of chamois population from Rodna Mountains between 1977-2013

More detailed information was obtained about the dynamic of individuals in population from Rodna Mountains by taking into account the spring and autumn observation in the breeding season (table 2, figure 2).

Table 2. The assessment of chamois in Rodna Mountains National Park in the period 2004-2013

No.	Year of assessment	No. of chamois in spring	No. of chamois in autumn
1	2004	36	61
2	2005	43	77
3	2006	64	109
4	2007	67	77
5	2008	117	140
6	2009	118	37
7	2010	134	126
8	2011	119	128
9	2012	132	159
10	2013	131	112

The figure 2 is showing a difference between assessments in spring and autumn. An important factor which is influencing the number of individuals is represented by the sheep and shepherd dogs from the mountains that are separating the herds and the chamois is very spread in the massif. Other factor which contributing to the dynamic of chamois is certain diseases (respiratory and digestive – ex. *Strongillus*) which is causing a high mortality especially for juveniles.

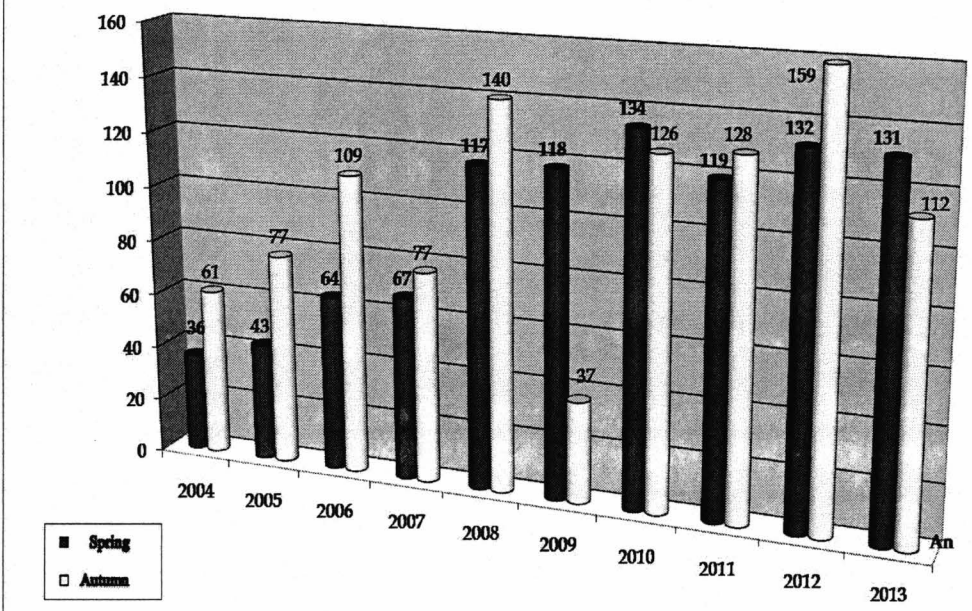

Fig. 2. Dynamic of chamois during 2004-2013 in Rodna Mountains National Park (two assessments per year)

Despite the fact that the chamois is not considered to be an endangered animal, or even an animal that is under threat from extinction, European ad national laws prohibits the hunting of the chamois inside of national parks order to try and conserve native mountainous animal species.

Rezumat

Capra neagră (*Rupicapra rupicapra carpathica*) este o specie ţintă pentru Parcul Naţional Munţii Rodnei (Rezervaţie a Biosferei). Studiul de monitorizare a dinamicii populaţiei s-a derulat pe o suprafaţă de 47.000 ha. Dinamica populaţiei de capre negre de-a lungul a 35 de ani dovedeşte oscilaţii numerice datorate factorului antropic, bolilor digestive şi respiratorii. Astăzi, populaţia este stabilă iar numărul indivizilor este în creştere.

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CONSIDERATIONS ON RISK FACTORS FOR MALIGNANT MELANOMA (MM)

Liana Monica DEAC*

Abstract: Malignant melanoma (MM) is the most dangerous type of skin cancer in the whole world and its incidence has increased in all ages of population in the recent decades. In Transylvania – (Romania) - the incidence for the disease has increased in the last 2 years to 24 per 100,000. Therefore it is necessary to develop programs which can reduce the morbidity and mortality of malignant melanoma. A retrospective epidemiologic study was conducted between 2009-2011 at 250 persons (18-70 age with malignant melanoma to identify the most important environmental risk factors for the illness. Subjects were interviewed face-to-face in their homes. The roles of constitutional factors and benign nevi in causation of malignant melanoma were also examined. Malignant melanoma was most common in people over 40. In ages younger than 45, women had higher incidence rates than men. High, intermittent exposure to solar UV was a significant risk factor for the development of malignant melanoma. Tanning beds, represented a risk which also increased the cancer incidence with 75%, no matter age, genetic makeup or gender. People at 35 or younger who used the beds regularly had a risk eight-fold higher than people who never used tanning beds. Occupational exposure to radiation and some chemicals (vinyl chloride, and petrochemicals) were associated with increased risk for the disease. People who worked or spend lots of leisure time outside were at increased risk too. Also persons who lived at higher elevations (mountain regional parts) were also at greater risk because the atmosphere is thinner and does not filter UV rays as well. A role for heredity in determining melanoma risk is suggested by familial concurrence of the disease. Epidemiological data strongly implicate sunlight as the principal environmental cause of malignant melanoma. Exposure to high levels of sunlight and the sunburns double the risk of developing the disease. Wearing of protective clothing can block out UV rays as well, adaptation of certain life-style, can prevent the disease morbidity also next.

Keywords: malignant melanoma, risk factors.

Introduction

It has been almost 200 years since Parisian physician Rene Laennec's first report of melanoma in Europe. Melanoma is a malignant tumor of pigment cells (Abbasiri, 2008), called melanocytes, which are found predominantly in skin but also in the bowel and the eye and it is due to uncontrolled growth of these and accounts for 75 percent of all deaths associated with skin cancer (Lachiewiicz, 2008, Markovick, 2007). Malignant melanoma (MM) is the most dangerous type of skin cancer in the whole world and its incidence has increased in all ages of

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population in the recent decades (Clinical Practice guideline for melanoma, 2008, Deac, 2010). For every 100,000 people in the United States, 17.7 new cases of melanoma are diagnosed each year (Pennie, 2007). In Transylvania – Romania, the incidence for the disease has increased in the last 2 years to 24 per 100,000. Therefore, it is necessary to develop more effective public health and earlier diagnosis programs, which can reduce the morbidity and mortality of malignant melanoma.

Material and Methods

A retrospective epidemiologic study was conducted between 2009-2011 at 250 persons from Transylvania, 138 female, 112 men, on 18-70 age, with malignant melanoma, to identify the most important environmental risk factors for the illness. Subjects, 195 with Urban environment and 55 with Rural environment, were interviewed face-to-face in their ambient place. The roles of constitutional and genetic factors and benign nevi in causation of malignant melanoma were also examined. There were identifies several MM, as: skin, eyes, brain, finger, ears and other. There were observed all possible signs of malignant melanoma with changes in a mole or pigmented area, in size, shape, height, or color analyzed. In some cases, there were observed irregular edge or border, itching or bleeding. Asymmetry, two sides of a mole looking or shaped differently or new moles growing near an existing mole were considered other interpretable signs in this study.

Results and Discussions

Incidence rates for MM. Malignant melanoma was most common in people over 40, although it affected children, young and middle-aged people as well, but the average age at diagnosis was 57 years of age. Incidence rates were especially high among people aged 65 years and older, group which made up 35% of melanoma cases (figure 1).

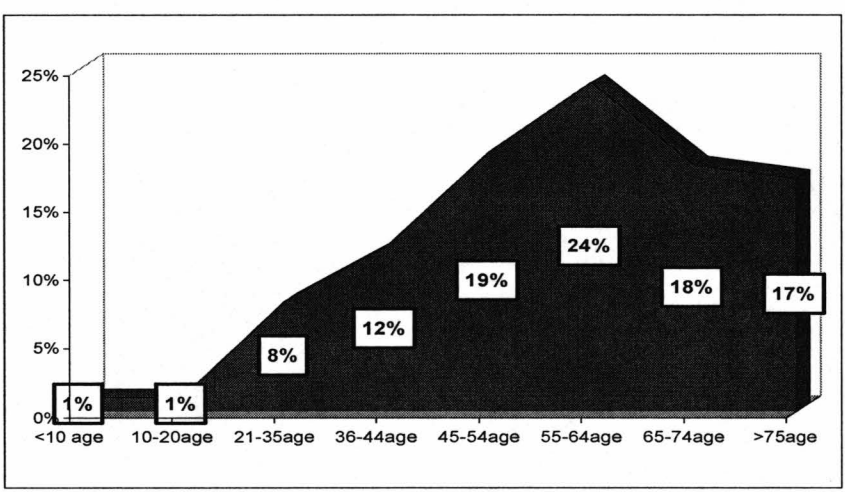

Fig. 1. Age incidence for MM

In ages younger than 45, women had higher incidence rates than men. Women and men have had similar rates in the age group of 45–54, but beginning at age 55, the incidence rates for men were greater than those for women.

Predilection of MM locations. The most common locations for malignant melanoma in women and men were on several anatomical skin parts (figure 2 and figure 3). For female mostly appeared on trunk and legs as on arms or other parts and for men it was present most dispread already.

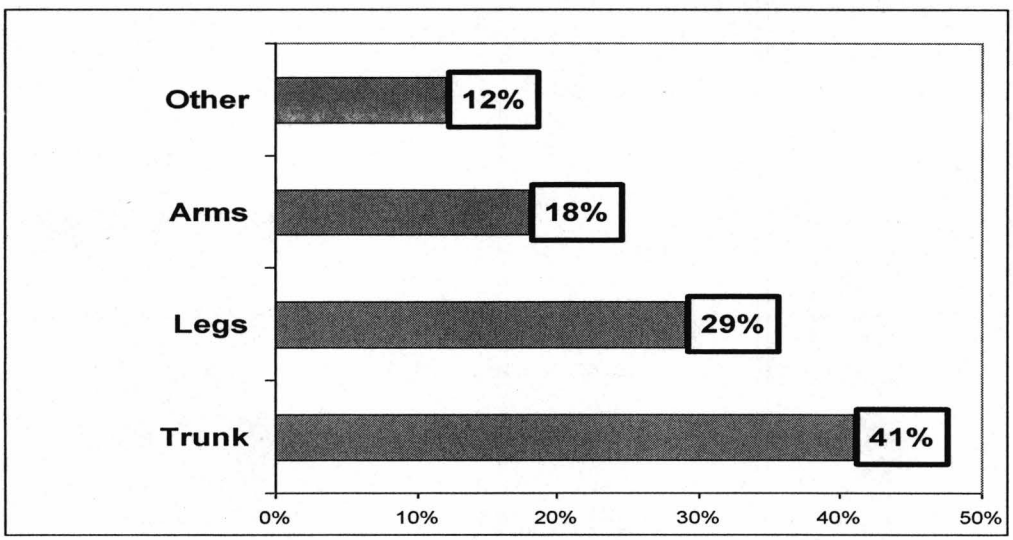

Fig. 2. Incidence of anatomical location at female

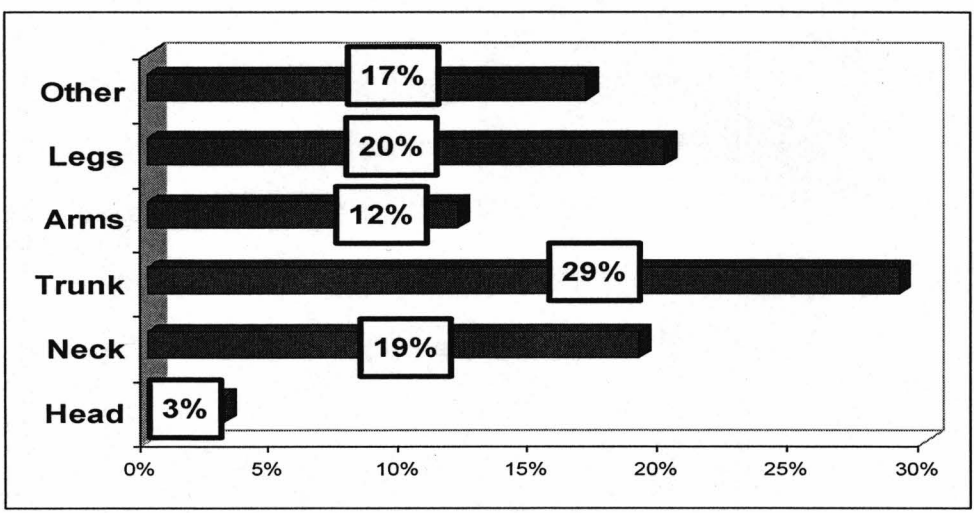

Fig. 3. Incidence of anatomical location at men

Presence of several risk factors. There were sought even the risk factors which determined the MM appearances. In this order, most susceptible phenotype characteristics were: *fair skin, red hair, blond hair and blue eyes* (figure 4).

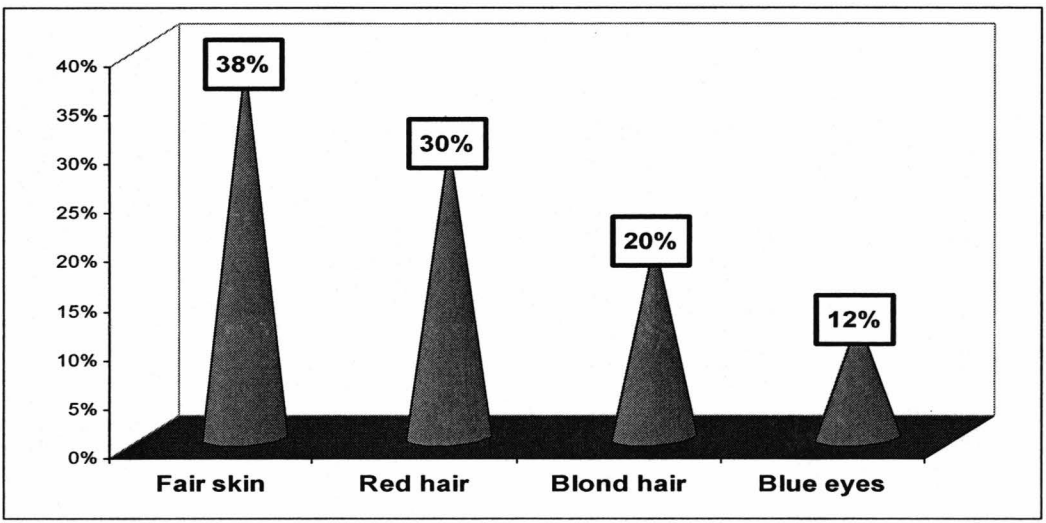

Fig. 4. Most phenotype and genotype characteristics

People with a prior history of dysplasia nevi (non-cancerous moles) and a family history of malignant melanoma have had almost 50% bigger risk for developing that disease too (figure 5). Most cases appeared when their relatives have had MM also.

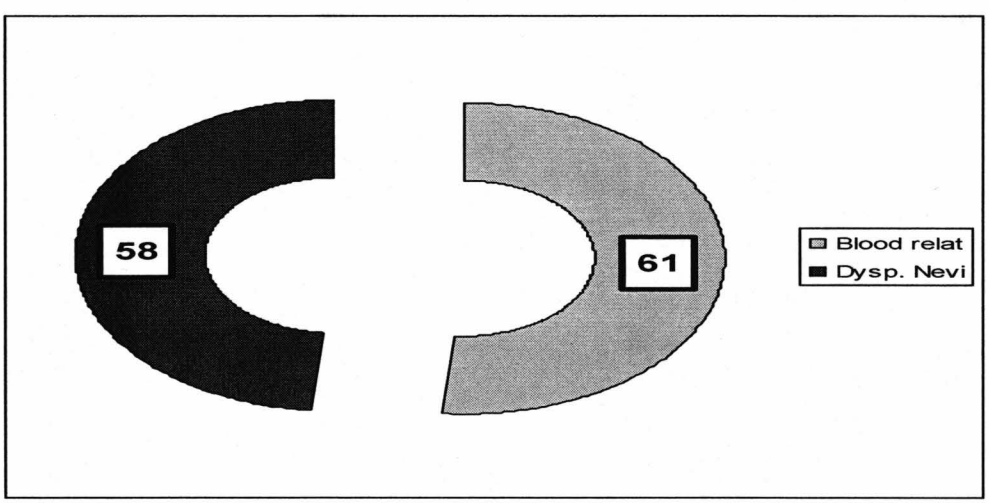

Fig.5. Family history for MM

High, intermittent exposure to solar UV, was a significant risk factor for the development of malignant melanoma and UVB was considered to represent the most carcinogenic one. Sunburn doubled the risk for developing the disease and tanning beds, exposure have increased that skin cancer incidence with 75% more (figure 6).

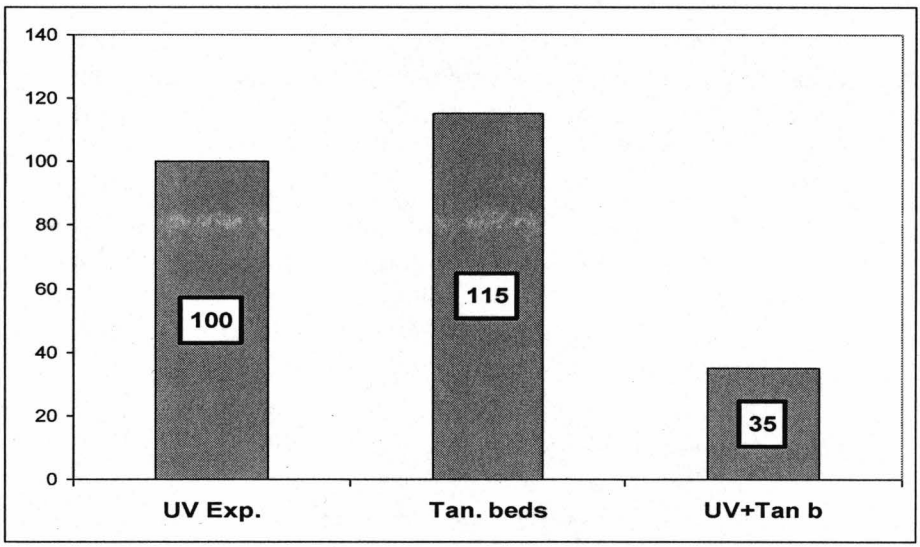

Fig. 6. Sun and tanning beds exposures

Occupational exposure to radiation and some chemicals as *vinyl chloride* and *petrochemicals* were associated with increased risk for the disease, in 74 cases (figure 7).

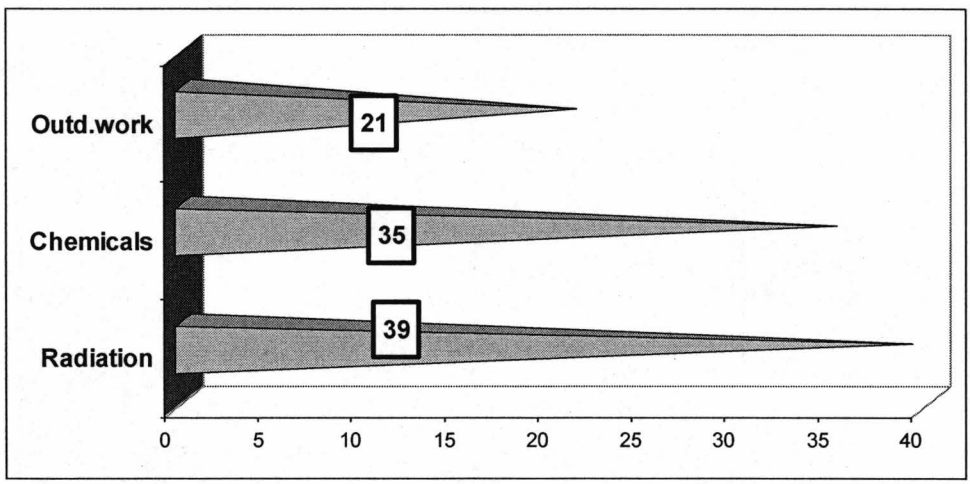

Fig.7. Occupational risk exposures for MM

Furthermore people who worked or spend lots of leisure time outside were at high increased risk too and the one who lived at higher elevations, as in mountain regional parts, were also at 2 % bigger risk, because the atmosphere was thinner and does not filter UV rays as well.

Data present that damaging life conditions with sun exposure for UV, sunburn, tanning beds use and life in mountain region comply with higher incidence for malignant melanoma cases, as any either risk presence as occupational exposure, family exposure, nevi presence but at same high risk value, as the phenotypic and individual characteristics represented by the disease in their family, skin, eyes or hair color aspects.

Prevention of disease up to, Centers for Disease Control and Prevention - CDC recommendations (Clinical practice guideline for melanoma, 2008, Lange, 2007, Lautenschlager, 2007, Gallather, 2005). Because of the risk for malignant melanoma even in children age, the CDC recommends to prevent the disease, with these guidelines for schools:

- Policies that reduce UV exposure
- Prevent environmental changes that exposure to the sun
- Educational tools to prevent cancer
- Familial commitment to improve prevention tactics
- Professional training programs for teachers and school administrators
- School health services to support prevent measures
- Evaluation of policy implementation

Today some protective measures and recommendations can have a major role, in decreasing the morbidity for malignant melanoma, all being effective public health care recommendations, as:

- wearing of protective clothing who can block out UV rays as well as adaptation of certain life-style changes;
- use of sunscreens or sun-block (that block out both UVA and UVB radiation) is well recommended;
- for anyone over the age 1, sunglasses should be worn to block all UV rays when in the sun.

Conclusions

Epidemiological data strongly implicate sunlight as one of principal environmental cause for malignant melanoma cases.

Exposure to high levels of sunlight and the sunburns or tanning beds use and life in mountain region double the risk of developing the disease.

Other risk factors as phenotype and individual characteristics, as disease present in their family, nevi, skin, eyes or hair colors, were also on high incidence.

Clinically, any suspicious skin lesion, should be checked immediately, especially if it has grown quickly or is partially raised.

Rezumat

Melanomul malign (MM) este în întreaga lume, cel mai periculos tip de cancer al pielii şi incidenţa sa a crescut în ultima vreme, peste tot şi la toate grupele de vârstă. În Transilvania – România, incidenţa îmbolnăvirilor a crescut în ultimii 2 ani, la 24 % de mii locuitori. Cunoscându-se acestea, este necesar a se dezvolta programe de sănătate publică eficiente, dar şi diagnostice cât mai precoce de îmbolnavire, pentru a scădea morbiditatea şi mortalitatea prin melanom malign. Între 2009-2011, am efectuat o evaluare epidemiologică retrospectivă, pentru 250 cazuri de melanom malign, legat de: semnele de îmbolnăvire, caracteristici şi particularităţi locale şi generale, antecedente heredo-colaterale, păstrând mereu judicios, limitele necesare de confidenţialitate, în înregistrări, sau în prelucrările datelor ulterioare. Astfel, s-a discutat şi analizat: orice modificare de culoare, textură, volum sau suprafaţă a unei aluniţe suspecte. Rolul unor factori constituţionali dar şi prezenţa nevilor benigni au fost şi ei examinaţi, pentru a analiza posibile cauzalităţi pentru melanomul malign. Cel mai des s-a înregistrat această patologie la persoane peste 40 ani. La grupa de vârstă sub 45 ani, la femei, s-a înregistrat cazuistica cea mai numeroasă, în timp ce la grupa peste 55 ani, incidenţa MM, a fost mai crescută la bărbaţi. Expunerea îndelungată la UV, a fost un factor de risc semnficativ şi dovedit în dezvoltarea melanomului malign. Totodată expunerea îndelungată la razele solare, a crescut incidenţa acestor cancere cu 75%, indiferent de sex sau factor genetic prezent. Astfel, persoanele de 35 ani, sau mai tinere, care regulat se expuneau la soare, au avut risc mai crescut de îmbolnavire, faţă de cele care nu stăteau la soare. Apoi anumite munci cu expunere prelungită la razele de soare, au fost alt factor de risc recunoscut, pentru apariţia MM. Totodată cei care locuiau în zone cu altitudine mai crescută (regiuni muntoase), au avut risc crescut de îmbolnăvire, datorită lipsei din aceaste zone, a filtrării razelor UV. Un rol considerabil pentru patologia aceasta a avut şi ereditatea. Datele epidemiologice susţin că în producerea melanomului malign, rolul principal îl are expunerea prelungită la razele de soare, care dublează cazuistica. Purtarea unor protecţii caracteristice prin îmbrăcăminte, pot bloca riscul la UV, ca şi adaptarea unor condiţii îmbunătăţite de igienă de viaţă, care pot preveni şi ele, apariţia acestor îmbolnăviri.

Cuvinte cheie: melanom malign, factori de risc.

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IMPORTANCE OF NORMAL MAGNESIUM LEVEL IN OUR BODY

Liana M. DEAC*, Nicoleta MUNTEAN*, Livia ALHAFEZ*

Abstract: Magnesium is a vital element for humans, plants and animals. Magnesium participates in numerous life-essential processes that occur both inside and outside the cells. This mineral is found in high concentrations inside cells, particularly those of the brain and heart. It is involved in more than 300 essential metabolic reactions. Normal serum magnesium range is: 1.7-2.4mEq/liter. The average daily intake of magnesium is estimated to be 200-300 mg/day, about 100 mg of which is absorbed. Most quantity of magnesium is absorbed in the small intestine. In a normal person, around 50 percent of dietary magnesium taken is absorbed. However, this depends on the concentration in the diet. Magnesium absorption requires an acidic stomach environment. Absorption is reduced by laxative and antibiotic abuse, antacids and alcoholics and those who are taking diuretics, are particularly at hypomagnesaemia risk, caused by the large urinary magnesium losses. The kidney is the main regulator of blood concentration and total body content of magnesium. Magnesium deficiency affects all the body tissues and makes appear a lot of significant symptoms and even may play a role in a number of disorders, as: cardiovascular, hypertension, stroke, early ageing. It also can results in impaired insulin secretion and reduces tissue sensitivity to insulin. Magnesium is vital for normal bone function and deficiency may contribute to osteoporosis. Most people may not get sufficient magnesium in their daily diets. As we get older we become more deficient in magnesium and therefore require more in our diet and in supplement form. Persons with low magnesium levels can be advised to eat magnesium-rich foods. To overcome magnesium deficiency magnesium supplements are used, available in a variety of forms in our pharmacies. Correct meals can prevent today the diseases caused by hypo and hyper-magnesium in our population.

Keywords: magnesium, deficiency, magnesium supplements.

Introduction

The French surgeon Pierre Delbet (1861-1957) considered magnesium as a sort of panacea, being able to play the role of elixir vitae in preventing all the hazards of senility (Agusz, 1996). That theory cannot be accepted anymore. Still today magnesium is a crucial element needed in our body to function on a daily normal physiological basis. This element represents the eighth most abundant mineral on earth, and the third most abundant in sea water. It is the

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fourth most abundant mineral in the body and the most abundant intracellular divalent cation, with essential roles in many physiological functions, as: helps maintain normal muscle and nerve function and keeps a steady heartbeat and it helps to build a healthy immune system. Adequate magnesium is a fundamental requirement for optimum function of the cardiovascular system, the nervous system and skeletal bones and muscle, as well other body tracts. That for magnesium deficiency can affect health of the heart, bones and blood vessels and alter blood sugar balance. Antibiotics, antidepressants, estrogen and heart drugs can all affect magnesium levels and diuretics are a major cause of magnesium deficiency.

Study objectives

It will be presented most characteristics and aspects concerning magnesium (Mg) as: Lab. determinations, physiology, role and functions of the mineral in the body to maintain our healthy condition and even how to treat, or how prevent us against several disorders, who can be caused by the modified its serum level.

Results and discussions

Laboratory Mg measuring methods. The Mg blood serum level can be measured by using atomic absorbance spectrophotometer (AAS). The acceptable value for Mg is considered the optimal serum range between 1.7 and 2.5 mg/ml, or 0.7-0.9 mmol, or 1.4-1.8 mEq/L. In spite of intense research activities there is still no simple, rapid, and accurate laboratory test to determine total body Mg status in humans. However, serum Mg < 0·75 mmol/l is a useful measurement for severe deficiency, and for values between 0.75; 0.85 mmol/l a loading test can identify deficient subjects. Plasma concentration is a reflection of the dietary intake of magnesium and of the ability of the kidneys and gastrointestinal tract to retain it. Because most magnesium is found intracellular, the relationship between total body deficiency and plasma concentration is poor. Other cellular Mg measurements, such as total or ionized Mg, frequently disagree and more research and systematic evaluations are needed. Muscle Mg appears to be a good marker, but biopsies limit its usefulness, as is the case with bone Mg, the most important but heterogeneous Mg compartment. The development of new and non invasive techniques such as nuclear magnetic resonance (NMR) may provide valuable tools for routinely analyzing ionized Mg in tissues. With the development of molecular genetics techniques, the recent discovery of Transient Receptor Potential Melastatin channels offers new possibilities for the sensitive and rapid evaluation of Mg status in humans. Unfortunately, the ideal test - tissue biopsies to assess intracellular magnesium content directly - is not practical. Total serum magnesium is normal present in three different states (figure 1). Less than 1 percent of the total body magnesium is present in blood. Thus, blood serum

measurements of magnesium that are routinely made in a clinical setting assess only a small part of the total magnesium stores in the body and magnesium in the blood does not necessarily correlate with the amount of magnesium in other parts of the body. At present there is little information about the state of magnesium within body pools and deficiencies are difficult to be point out today.

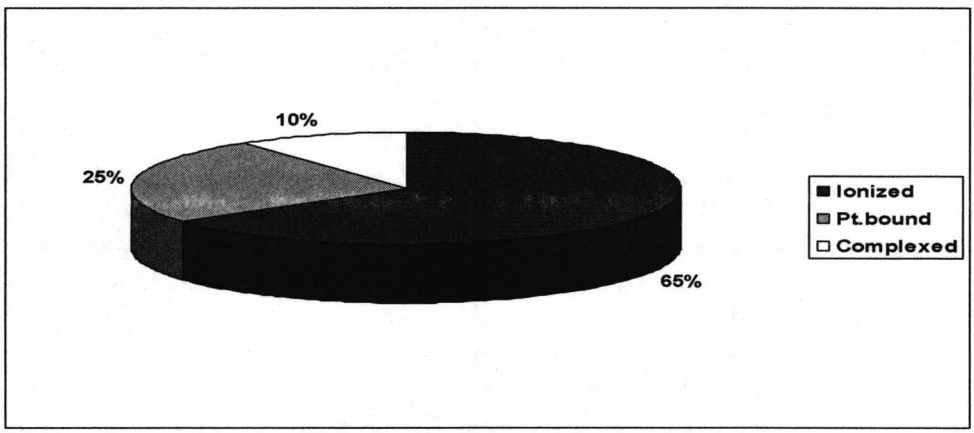

Fig. 1. States of Magnesium (65% is ionized, 25% is bounded to proteins and 10% exist in several organically complexes)

Physiology of magnesium. Magnesium plays important roles in the structure and the function of the human body. The adult human body contains about 25 grams of magnesium. Over 60% of all the magnesium in the body is found in the skeleton, about 27% is found in muscle, 6% to 7% is found in other cells, and less than 1% is found outside of cells. The adult body contains about 20 to 28 g of magnesium with about 60 percent of this present in the bones, and the rest in the muscle, soft tissue and body fluids. On average, the body contains only 24 grams of magnesium; 99% is within the cells, particularly those of the brain, heart, and skeletal muscle. When magnesium levels are low, problems can arise. Magnesium is a co-factor in over 300 enzyme reactions, particularly those involving the metabolism of food components and the formation of new compounds essential for good health (Alexander et al., 2008). All enzymatic reactions requiring the energy storage molecule, adenosine triphosphate (ATP), require magnesium. It is also for protein synthesis, DNA manufacture, fatty acid synthesis, anaerobic breakdown of glucose; and the removal of toxic substances, such as ammonia, from the body. Magnesium is involved in the maintenance of the membrane electric potential and the transport across membranes of sodium, potassium and calcium. Magnesium is also involved in nerve impulse transmission and it is necessary for the action of a compound which plays a vital role in transmitting messages from hormones

and other stimuli which cause chemical reactions inside cells. In contrast with other ions, magnesium is treated differently in 2 major respects: first bone, the principal reservoir of magnesium, who does not readily exchange with circulating magnesium in the extracellular fluid space and second only limited hormonal modulation of urinary magnesium excretion. The inability to mobilize magnesium stores means the negative magnesium balance. Concerning the absorption and excretion, magnesium is mostly absorbed in the small intestine, through a saturable transport system and via passive diffusion through bulk flow of water. Absorption of magnesium in a normal person depends on the amount ingested. When the dietary content of magnesium is typical, approximately 30-40% is absorbed. However, this depends on the concentration in the diet, with a lower percentage absorbed from a high magnesium diet. Magnesium absorption requires an acidic stomach environment. Absorption is reduced by laxative abuse, infections and allergies. Foods low in protein or high in phosphorus can reduce magnesium absorption. Magnesium balance, like that of other ions, is a function of intake and excretion. The kidney is the main regulator of blood concentration and total body content of magnesium (Quamme, 1997). Excretion mainly occurs at night. High protein and high sugar diets may increase magnesium excretion. In the healthy adult, there is no net gain or loss of magnesium from bone so that balance is achieved by the urinary excretion of the approximately 100 mg (4.1 mmol) that is absorbed. Changes in intake are balanced by changes in urinary magnesium reabsorption, principally in the loop of Henle and the distal tubule (Wagner, 2007). Magnesium balance must work always very well for to offer a normal intake and urinary/ fecal output. The main controlling factors in magnesium homeostasis appear to be gastrointestinal absorption and renal excretion.

Functions of Magnesium in human body. Magnesium is the second most prevalent electrolyte in the body, and has a role in the human body as an essential dietary mineral. Magnesium is the key mineral involved in the enzymatic process of converting food to energy. It is also essential for both the synthesis and secretion of insulin (Montagnana et al., 2008). Magnesium allows blood vessels to relax and dilate, encouraging normal blood flow to the brain. Magnesium is tied very closely to adrenal processes and particularly the manufacturing of stress hormones. Insufficient levels of magnesium in the body can result in over-production of stress hormones such as cortisol, resulting in symptoms of anxiety and mood swings. Magnesium is one of the beauty minerals, helping to maintain skin elasticity and dermal protection. low levels can lead to skin problems, poor healing and complexion. As an essential dietary mineral, magnesium plays many important roles, including: acts as a co-factor for metabolic enzymes, assists energy production in cells, supports nerve and muscle function, helps maintain a normal, regular heartbeat, supports bone

density, it promotes normal blood pressure, is known to be involved in energy metabolism.

Etiological aspects of magnesium deficit risks. Primary magnesium deficit , originates from two mechanisms: deficiency and depletion (Druke, 2007). Primary magnesium deficiency, is due to insufficient magnesium intake. Primary magnesium depletion, is due to deregulation of factors controlling magnesium status, as intestinal magnesium hypo absorption, reduced magnesium bone uptake and mobilization, sometimes urinary leakage, decreased adaptability to stress, insulin-resistance and adrenergic hypo receptivity. Secondary magnesium deficit is from depletion. It largely results, from various illnesses and treatments common to elderly persons. Stress can be a cause of magnesium deficiency, and a lack of magnesium tends to magnify the stress reaction, worsening the problem. Little or no physical activity can lower Mg body level. Additionally, drugs like birth control pills, hypertension medicine, diuretics, insulin, and certain antibiotics deplete magnesium levels. Sweating often from exercise or other causes can also deplete magnesium. Dietary factors can also deplete Magnesium when it is: consumption of much caffeine, consumption of much sugar, consumption of processed food, consumption of alcohol, consumption of produce from depleted soil, consumption of foods high in phytic acid. People most at risk of magnesium deficiency include: the elderly, diabetics, children, those on low calorie diets, those over-indulging on alcohol, those engages in heavy exercise, those who have stressful lifestyles.

Epidemiology. Although the incidence of hypomagnesaemia in the general population has been estimated at less than 2%, some studies have estimated that 75% of Americans do not meet the recommended dietary allowance of magnesium (Guerrero et al., 2002). In these (figure 2) it have been reported that hypomagnesaemia occurs in 10-20% of hospitalized patients and 50-60% of patients in intensive care units. It is also very common in alcoholics (30-80%) and it occurs in 25% of diabetic outpatients.

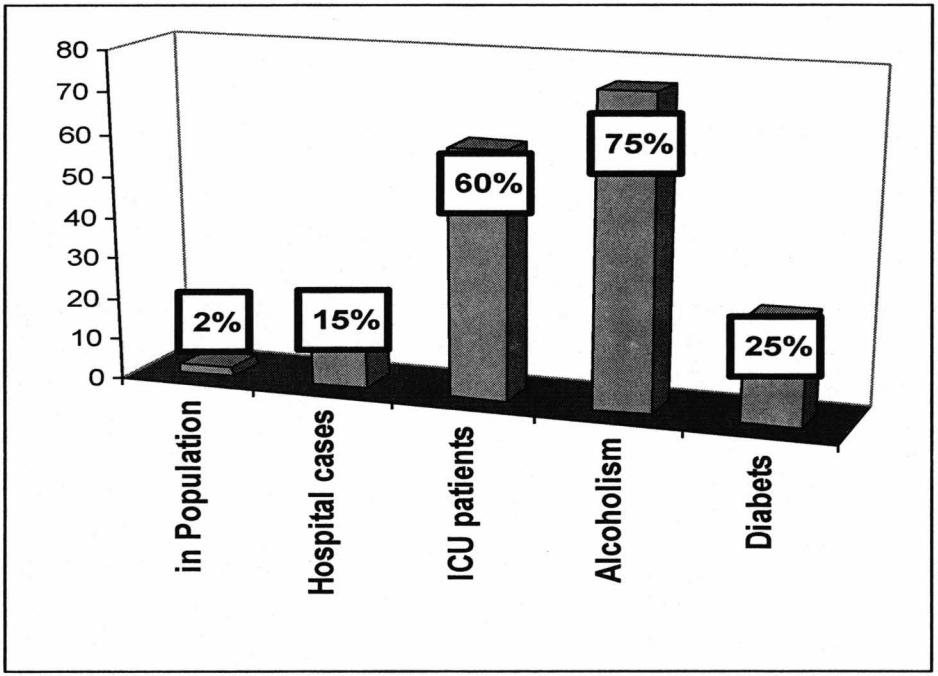

Fig. 2. Incidence of hypomagnesaemia (in general in population hypomagnesaemia is almost 2% and particular hypomagnesaemia is a common entity, occurring almost in: 15 % in hospitalized patient cases, 60 % in patients hospitalized in an intensive care setting- ICU cases and it can coexist at 25% diabetic cases, or at 75% of alcoholics' patients) (Whang et al., 1990).

Hypomagnesaemia is a common entity occurring in up to 12 percent of hospitalized patients. The incidence rises to as high as 60 to 65 percent sometimes in patients in an intensive care setting in which nutrition, diuretics, hypoalbuminemia, and amino glycosides may play important roles (Whang et al., 1990). Hypomagnesaemia considered a medical emergency when Magnesium is <0.30 mmol/L. Epidemiological studies show that death rates from coronary heart disease are higher in areas where the water is low in magnesium. Magnesium deficiency is especially prevalent in elderly people. This is due to low dietary intakes and also to the decreases in absorption and increases in excretion associated with aging. Stress can be a cause of magnesium deficiency, and a lack of magnesium tends to magnify the stress reaction, worsening the problem. Additionally, drugs like birth control pills, hypertension medicine, diuretics, insulin, and certain antibiotics (among others) deplete magnesium levels. Sweating often from exercise or other causes can also deplete magnesium. Epidemiological evidence suggests that a low intake of magnesium is associated with impaired lung function, bronchial hyper reactivity and wheezing. Hypomagnesaemia inhibits the precipitation of

calcium phosphate and calcium oxalate, two substances which contribute to the formation of kidney stones.

Symptoms of Magnesium deficiency. These develop over time. Marginal magnesium deficiency is considered to be very common and may affect 15 to 20 percent of the population and is common in those who eat diets high in processed foods, alcoholics, and in those with malabsorption problems. Most common signs for hypomagnesaemia are: low energy levels and tiredness, rapid exhaustion, excessive need to sleep, cold feet, numbness in hands and feet, sensitivity to noise, anxiety, restlessness and irritability (inner restless), weakness, anorexia or loss of appetite ,migraines and headaches, depression, muscle cramps and tics, irregular heartbeat (palpitation, tachycardia),clogged arteries, high blood pressure, insulin resistance. An ongoing magnesium deficiency can lead to more serious symptoms, including: numbness and tingling, muscle contractions and cramps, seizures, personality changes, abnormal heart rhythms, coronary spasms (Tong et al., 2005). It has recently been theorized that magnesium deficiency may contribute to accelerated aging, through effects on the cardiovascular and nervous systems (Deac, 2010), as well as muscles and the kidneys modifications.

Diseases caused by Magnesium deficiency. Inadequate magnesium intake has been linked to several types of: cardiovascular disease, blood pressure, heart attack, angina, ischemic heart disease and cardiac arrhythmias, atherosclerosis. Studies suggest that around 30 percent of high blood pressure sufferers consume inadequate amounts of magnesium and high blood pressure is more common in areas where the water is low in magnesium (Herbert, 1996). Cardiac arrhythmias appear at low serum magnesium level, because this mineral maintains potassium concentrations inside of muscle cells. Low blood magnesium levels are commonly associated with many *complications of diabetes*, including heart disease and high blood pressure. Low dietary intake also was linked to lower beneficial HDL cholesterol levels (Rashed et al., 2012). Cholesterol may be more susceptible to oxidative damage when magnesium levels are low. Magnesium deficiency may impair vitamin D metabolism which adversely affects bone-building. That for maintaining normal the magnesium balance is very important in the prevention of osteoporosis.

Magnesium supplementation and treatment recommendations. Some nutrition studies have shown that most people may not get sufficient magnesium in their daily diets. It has been recommended a daily intake of 6 to 10 mg per kg of body weight per day for optimal health. Many kind of magnesium supplements are used all over the world today, as magnesium oxide, magnesium carbonate, chelated magnesium (magnesium glycinate), magnesium orotate, magnesium citrate, magnesium maleate, and magnesium

gluconate. We recommend from all these the Magnesium orotate products, for their better source of magnesium and because of their improved absorption. Vitamin B6, vitamin D, and selenium can be additionally given, to help the Mg absorption in therapy, along with some daily meals including green vegetables, fresh/dried fruits, cacao derivatives, wheat, and seafood, acknowledged as Mg abundant natural products. Good sources of magnesium include whole grains, nuts, soybeans, avocados, beans, corn, lemons and dark green leafy vegetables, as magnesium forms part of the green pigment, chlorophyll. Magnesium is ubiquitous in nature and is especially plentiful in green vegetables, cereal, grain, nuts, vegetables. Meats and milk have an intermediate magnesium content, while refined foods generally have the lowest magnesium content, but meat also contains calcium, phosphate and protein which reduce the amount of available. Unrefined grains and nuts also have high magnesium content. Refined foods generally have the lowest magnesium content. Flour refining, rice polishing, sugar extraction from molasses and other methods of food processing remove almost all the magnesium from these foods. Food processing and cooking may deplete magnesium content, thus accounting for the apparently high percentage of the population whose magnesium intake is less than the daily allowance. Drinking water is an important source of magnesium, especially in hard water areas, and is usually better absorbed than magnesium from food. Although only about 20-50% of the magnesium intake is actually absorbed by the body Magnesium is also available in many therapeutically forms. Treatment for hypomagnesaemia depends on the degree of deficiency and the patient's clinical symptoms and signs. Therapy can be oral for patients with mild symptoms or intravenous for patients with severe symptoms or those unable to tolerate oral administration. Recommended types include magnesium citrate, magnesium gluconate, and magnesium lactate, all of which are more easily absorbed into the body than other magnesium salt forms. Dosages are based on the dietary reference intakes. The Food and Nutrition Board (FNB) from the Romanian Minister of Health, have already set the tolerable upper intake level (UL) for magnesium at 350 mg/day. This UL represents the highest level of daily supplemental magnesium intake likely to pose no risk of diarrhea or gastrointestinal disturbance in almost all individuals. The FNB cautions that individuals with renal impairment are at higher risk for adverse effects from excess supplemental magnesium intake and also notes that there are some conditions that may warrant higher doses of magnesium under medical supervision (figure3). Magnesium requirements are increased during rapid growth in children and adolescents (Knoers, 2009). Older adults are less likely than younger adults to consume enough magnesium to meet their needs and should therefore take care to eat magnesium-rich foods in addition to taking a multivitamin-mineral supplement daily. Because older adults are more likely to have impaired kidney function, they should avoid taking more than 350 mg/day of supplemental magnesium without medical consultation. Modern

food production has reduced the average magnesium intake from 400 mg per day to 300 mg per day over the last 70 years (Ryder et al., 2009).

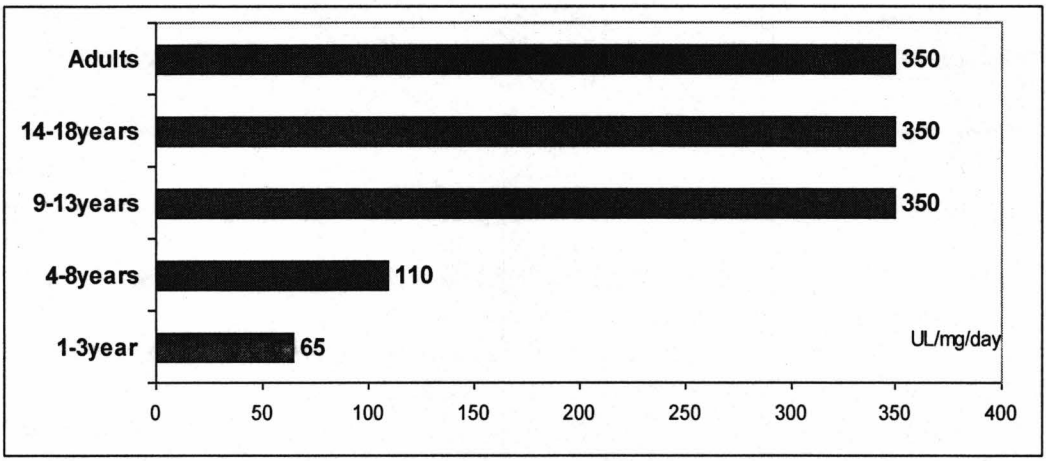

Fig. 3. Tolerable Upper Intake Level (UL) for Supplemental Magnesium

A person's need for magnesium increases during pregnancy, recovery from surgery and severe illnesses, heavy working and athletic training. That for workers may need even 400 UL/mg/day and breastfeeding females only 320 UL/mg/day. Because of the potential for side effects and interactions with medications, dietary supplements should be taken only under the supervision of a knowledgeable medical adviser.

Toxic effects of excess Mg intake. Adverse effects from excess magnesium have been observed with intakes of various magnesium salts Magnesium toxicity is rare as the body excretes excess. Symptoms of toxicity include diarrhea, flushing of the skin, thirst, low blood pressure, loss of reflexes, lethargy, weakness, fluid retention, nausea, vomiting and shallow breathing. The most common cause of excess magnesium is renal failure. Elevated serum levels of magnesium named hyper-magnesaemia, may result in a fall in blood pressure-hypotension. Some of the later effects of magnesium toxicity, such as lethargy, confusion, disturbances in normal cardiac rhythm, and deterioration of kidney function, are related to severe hypotension.

Patient Education. Patients should be counseled regarding modification of risks of magnesium levels. Such modifications may include maintaining a proper diet, ceasing alcohol consumption, improving diabetic controls, and taking supplements if the cause of hypo, or hyper-magnesaemia is still present.

Conclusions

Every single cell in the human body demands adequate quantities of magnesium to function, otherwise will die.

Magnesium deficiency can affect the health of the heart, bones, and blood vessels and alters blood sugar balance.

Magnesium deficit largely depends on the variety of pathological conditions, nutritional deficiencies, and medical treatments.

Because magnesium plays important roles in blood vessel structure and function (by maintaining endothelial and platelet integrity), a deficiency can increase progression of arteriosclerosis, hypertension, and cardiac complications.

The ageing condition indirectly influences the magnesium deficiency and this can determine the clinical pattern of ageing, by selectively affecting the neuromuscular, cardiovascular, and other apparatuses or systems.

Correct daily intake, good contain of Mg food and magnesium supplements, can help to maintain the normal serum balance and with to prevent illnesses caused by hyper or hypo level of this mineral in our body.

Rezumat

Magneziul este un element vital pentru: om, animale şi plante. El participă în numeroase procese esenţiale ale vieţii, ce se derulează ades intra sau extracelular. Acest mineral se găseşte în concentraţie crescută în celule, în special în cele ale creierului şi inimii. Magneziul normal în ser este de 1.7-2.4 mEq/litru. Media de consum utilă pe zi este de 200-300 mg, din care doar 100 mg se şi absoarbe în organism. La persoane normale se absoarbe cam 50% din aportul zilnic de magneziu. Mediul acid din stomac facilitează absorbţia acestuia. Absorbţia este redusă la un consum în exces de laxative şi antibiotice, dar şi de cel de antiacide, sau de alcool, iar aceia care mai iau şi diuretice, se află la risc de hipomagnezemie vădit, cauzat de pierderile mari prin urină ale mineralului. Rinichiul este acela care reglează concentraţia de magneziu. Deficitul de magneziu afectează toate ţesuturile şi face posibilă apariţia unor multiple simptome ulterioare, dar şi joacă un rol major în producerea unor dezordini organice: cardiovasculare, hipertensiune arterială, atac cerebral, îmbătrânire precoce. Totodată, deficitul mai poate genera modificări ale nivelului insulinei din corp. Magneziul este vital pentru funcţionalitatea normală a oaselor, iar deficienţa contribuie la apariţia osteoporozei. Majoritatea populaţiei nu işi ia necesarul de magneziu din masa zilnică, apoi cu cât înaintăm în vârstă, creşte şi deficitul de Mg în organism, ceea ce implică necesitatea unor administrări de suplimente nutritive, care să conţină negreşit acest mineral în masa lor zilnică. Persoanele cu un nivel scăzut al magneziului în sânge, vor fi îndrumate să consume mai multe produse alimentare bogate în acesta. Pentru a depăşi deficitul de magneziu din organism, există multiple produse farmaceutice, din gama suplimenţilor nutritivi. Hrănirea corectă poate preveni apariţia unor îmbolnăviri cauzate de hipo sau hipermagnezemie în populaţia noastră.

Cuvinte cheie: magneziu, deficit, suplimente nutritive.

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THE EFFECT OF TWO HYPERICUM SP HYDROALCOHOLIC EXTRACTS ADMINISTRATION ON SOME BIOCHEMICAL BLOOD PARAMETERS IN WHITE WISTAR RAT

Ioana ROMAN*

Abstract: The aim of experiment was to study the effects of two different alcoholic extracts, containing 0.3% and 0.15% hypericin, of two different species of Hypericum (*H. perforatum* L. and *H. maculatum* Crantz.) on some biochemical parameters in rats. Extracts were administered for 10 days in white female Wistar rats, weighing 200 ± 20 g, that were previously intoxicated with alcohol (6g/kg body) for 35 days. We found that the administration of Hypericum perforatum extract in a lower dose (0.15% hipericină/kg bw) had the most visible positive effects, respectively, had expressed hipolemiant effect by decreasing of triglyceride values in response to the presence of alcohol in the body, which caused injuries to hepatocytes.

Keywords: biochemical blood parameters, alcohol intoxication, hypericum perforatum, hypericum maculatum, rats.

Introduction

St. John's wort has a long history of folk use. Dioscorides, the foremost physician of ancient Greece, as well as Pliny (in ancient Rome) and Hippocrates (the father of medicine), administered St. John's wort in the treatment of many illnesses. In folk medicine, St. John's wort has been used in the treatment of wounds (it has powerful antibacterial and antiviral properties), kidney and lung ailments, and what we would now call depression.

With the current information, it is evident that *Hypericum sp.* has pharmacological functions including antidepressant, antioxidant, anticonvulsant, analgesic, anti-inflammatory, cytotoxic and antidiabetic activities, among others. As the current information shows, it is also possible that hypericin and hyperforin might be useful in the development of new drugs to treat various diseases (Asgarpanah, 2012).

Hypericum sp. (in folk medicine, St. John's wort, in romanian - sunătoarea), is currently being medically studied as a treatment for AIDS, several forms of cancer, bed wetting and night terrors in children, skin diseases such as psoriasis, rheumatoid arthritis, peptic ulcers, and even hangover. Several studies were performed in order to investigate the influence of *Hypericum* extracts on alcohol intake in animals (Coskun et al., 2006, Perfumi et al., 2005, Perfumi et al., 2005a, Rezvani et al., 2003). All studies report beneficial effects on ethanol

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withdrawal symptoms, additionally a reduction of alcohol intake in alcohol preferring animals was observed. *Hypericum* extracts and opioid receptor antagonists act synergistically (Perfumi et al., 2003).

The changes in the alcohol drinking behaviour may be caused by hyperforin (Perfumi et al., 2001, Wright et al., 2003). In contrast, De Vry et al., (1999) reported a reduction in alcohol preference after administration of an extract with very low hyperforin content. Clinical data are missing (Tayfun Uzbay, 2008).

A constituent chemical, hyperforin, may be useful for treatment of alcoholism, although dosage, safety and efficacy have not been studied (Kumar et al., 2006).

Currently, in Romania, attention of studies was directed on the effects of this plant in order to improve the nervous depression and less on other diseases, such as alcoholism.

Hence, in our study we have proposed to research the protective effect of some vegetal extracts derived from two species of Hypericum encountered more frequently in our country's flora, respectively Hypericum perforatum, known as officinal plant and Hypericum maculatum, in terms of under chronic alcoholic intoxication in rats.

Materials and methods

The experiment was made on mature white Wistar female rats, weighing 200 ± 20 g, divided into 6 experimental groups of 6 animals each, as follows:

- Control group, **C;**

- **Alc** group, treated for 35 days with ethilic alcohol 50° in amount of 6 g/kg, which was administered in drinking troughs, along with drinking water;

- **HyP1** group, which was treated for 25 days with alcohol 50° in amount of 6 g/kg. After this period animals were further treated for 10 days with a mixture consisting of tincture of *Hypericum perforatum* containing 0.3% hypericin / kg corp (675 mg dried subst.) in ethyl alcohol 50°, given by intragastric gavage for 10 days;

- **HyP2** group, which was treated for 25 days with alcohol 50° in amount of 6 g/kg. After this period animals were further treated for 10 days with a mixture consisting of tincture of *Hypericum perforatum* containing 0.15% hypericin/kg corp (340 mg dried subst.) in ethyl alcohol 50°, given by intragastric gavage for 10 days;

- **HyM1** group, was treated for 25 days with alcohol 50° in amount of 6 g/kg. After this period animals were further treated for 10 days with a mixture consisting of tincture of *Hypericum maculatum* containing 0.3% hypericin/kg corp (552.5 mg dried subst.) in ethyl alcohol 50°, given by intragastric gavage for 10 days;

- **HyM2** group, was treated for 25 days with alcohol 50° in amount of 6 g/kg. After this period the animals were further treated for 10 days with a mixture consisting of tincture of *Hypericum maculatum* containing 0.15% hypericin / kg

corp (276.24 mg dried subst.) in ethyl alcohol 50°, given by intragastric gavage for 10 days;

Animals were obtained from the biobasis of „Iuliu Haieganu" Medicine and Pharmacy University, Cluj-Napoca and kept under standardized zoohigienical conditions: range of light/dark 12:00; standardized diet, administered as a concentrated food (pelleted feed), balanced protein, carbohydrates, lipids and vitamins, drinking water and food were given „ad libitum", in accordance to the European Communities Council Directive 2010/63/UE Directive of European Parlament and according to the approval of the Ethics Committee and Animal Protection for Experiments from the Institute of Biological Research, NIRDBS branch, Cluj-Napoca, Romania (Decision 1/28.02.2013).

The alcoholic extract of Hypericum perforatum is a tincture 1:10, containing 0.0442 g flavonoids expressed in rutoside/10 ml tincture (4.421% rutoside) and 0.0044 g total hypericin /10 ml tincture (0.444% total hypericin).

The alcoholic extract of *Hypericum maculatum* is a tincture 1:10, containing de 0.0495 g flavonoids expressed in rutoside/10 ml tincture (4.957% rutoside) and 0.0054 g total hypericin /10 ml tincture (0.543% total hypericin).

On the 35th experimental day animals were sacrificed by decapitation after a previous anesthesia with ether.

The blood was colected and than processed according to analyzed functional parameters. In our study we kept track of some proteines, lipids and carbohydrates metabolic parameters, as well as indicators of renal function parameters. For all biochemical determinations (serum creatinine and urea, total protein - TP, triglycerides and cholesterol, blood sugar) blood samples were immediately centrifuged, serum harvested and then frozen in Eppendorf vials. Measurements were made with biochemistry semiautomatic analyzer screen point type, with reagents - STATE - FAX 1904 Plus, Global Medical Instrumentation, Inc. 6511 Bunker Lake Blvd Ramsey Minnesota, USA 55303.

Biochemical data were statistically processed by means of Student's „t" test. Aberrant values were eliminated by means of Chauvenet's criterion. A probability value of $p \leq 0.05$ was considered significant.

Results and Discussion

Serum level of cholesterol, triglycerides, total proteines (TP), urea, creatinine and blood glucose

From the analysis of the results given in table 2 we notice an increase of the TP values (42,67%) in Alc group, with 48,88% in HyP1 group, with 26,41 in HyM1 group respectively, 14.65 % in HyM2 group.

In HyP2 group, the values do not change significantly. These changes of the TP values are parallel and in the same sense as those of blood urea. The TP

value is significant changed both in the HyM1 and HyM2 groups which increase with 42,01 and 50,73%% respectively.

The Hypericum maculatum extract administration has led to an increase in the serum urea levels with 42.01% in HyM1 group respectively, with 50.73% in HyM2 gropup. The serum creatinine level s do not show any significant changes from the 6 experimental groups.

As regarding the cholesterol, this parameter shows no significant changes of values; triglycerides increase in the HyM2 group with 47,53%, probably due to the potentation of the alcohol effect on this parameter and decrease in the HyP2 group with 28,81%. In this group, we observe a more pronounced decrease of cholesterol values, with 8.27% against C group. Blood sugar levels do not suffer significant changes in treated groups versus the control group.

The values of serum biochemical parameters are presented in Table 1.

Table 1. Serum biochemical parameters

	M	Alc	HyP1 (0,3%)	HyP2 (0,15%)	HyM1 (0,3%)	HyM2 (0,15%)
Cholesterol (mg/dL)						
x ± ES	63,1±6,43	75,42±9,03	62,82±9,4	60,77±5,17	72,92±9,41	66,27±7,03
n	6	6	6	6	6	6
p	-	NS	NS	NS	NS	NS
D%	-	+19,53	-0,43	-8,27	+15,57	+5,03
Triglycerides (mg/dL)						
x ± ES	99,7±11,8	108,02±25,0	102,85±16,8	71,02±7,79	114,9±19,92	147,2±21,63
n	6	6	6	6	6	6
p	-	NS	NS	0,5<p<0,05	NS	0,5<p<0,05
D%	-	+8,27	+3,08	-28,81	+15,16	+47,53
T.P. (g/L)						
x ± ES	79,5±1,85	113,42±11,0	115,97±17,9	90,75±9,67	100,5±8,27	91,15±5,43
n	6	6	6	6	6	6
p	-	<0,02	0,5<p<0,05	NS	>0,05	0,5<p<0,05
D%	-	+42,67	+48,88	+14,15	+26,41	+14,65
Urea (mg/dL)						
x ± ES	22,07±0,2	20,42±0,82	22,8±4,1	28,1±3,13	31,35±7,14	33,27±2,7
n	6	6	6	6	6	6
p	-	NS	NS	NS	>0,02	<0,01
D%	-	-7,47	+3,28	+27,29	+42,01	+50,73
Creatinine (mg/dL)						
x ± ES	0,77±0,02	0,82±0,07	0,8±0,04	0,85±0,001	0,72±0,04	0,77±0,07
n	6	6	6	6	6	6
p	-	NS	NS	NS	NS	NS
D%	-	+6,45	+3,22	+9,67	-6,45	0
Blood glucose (mg/dL)						
x ± ES	147,75±4.9	140,25±4.36	140,5±5,33	152,5±3,92	147,75±6,9	159,25±9,1
n	6	6	6	6	6	6
p	-	NS	NS	NS	NS	NS
D%	-	-5,07	-4,9	+3,21	0	+7,78

Legend: Are given: percentage difference *vs.* the control group (±D %); the statistically significant values were considered to be for $p \leq 0.05$.

As regards the total protein values dynamics at the 6 experimental groups, it is known that in rodent liver, alcohol inhibits the secretion of newly synthesized glycoproteins and albumins secretion by hepatocytes. This is due to the binding of acetaldehyde to tubulin, affecting the microtubules of which depends on the secretion of proteins in the cell. In rats fed with alcohol the amount of protein that binds fatty acids increases, thus increasing the total cytosolic proteins. (www.doctor.info.ro/alcoolul_mecanismul afectari.html)

In addition to these changes we consider that has contributed and body dehydration rats. The experiment took place in summer, and the alcoholic rats, as well as the rats treated with extracts, presented more accentuated sleepiness have not hydrated enough, except HyP2 (0.15 % hypericine) group, to which was manifested the protective effect of the Hypericum perforatum extract compounds on the brain and liver, as we pointed out in our previous paper (Roman et al., 2011).

Hipocolesteremiant effect of the Hypericum extract might be due to its ability to decrease total cholesterol and serum triglycerides by reducing lipid peroxidation processe and increases of the antioxidant enzymes activity (Zou et al., 2005).

The level of both blood sugar and creatinine shows no significant serum changes in the 6 experimental groups. Urea level changes as a result of Hypericum maculatum vegetal extracts administration can be related to an imbalance of protein metabolism manifested through increased levels of total protein in these experimental groups.

Conclusions

In conclusion, we consider that in the case of blood biochemical changes at HyP2 group, administration of Hypericum perforatum extract in a lower dose (0.15% hipericină/kg bw) had the most visible positive effects, has expressed hipolemiant effect by decreasing of triglyceride values in response to the presence of alcohol in the body, which caused injuries to hepatocytes.

Rezumat

Scopul experimentului a fost de a studia efectele a două extracte alcoolice diferite, conţinând 0,3% şi 0,15% hipericină, de la două specii diferite de Hypericum (*H. perforatum* L. and *H. maculatum* Crantz.) asupra unor parametrii biochimici sanguini la şobolani. Extractele au fost administrate timp de 10 zile la şobolani albi Wistar, femele, în greutate de 200 ± 20 g, care au fost intoxicaţi în prealabil cu alcool (6g/kg greut. corp.) timp de 35 zile. Am găsit că administrarea extractului de Hypericum

perforatum la o doză mai mică (0.15% hipericină/kg bw) a avut efectele cele mai vizibile, respectiv, a prezentat un efect hipolipemiant prin scăderea valorilor trigliceridelor, ca răspuns la prezenţa alcoolului în organism, care a determinat leziuni ale hepatocitelor.

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AN OVERVIEW OF THE MOLECULAR COMMUNICATION BETWEEN NITROGEN-FIXING BACTERIA AND *FABACEAE* IN ESTABLISHING THE SYMBIOSIS

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Abstract: The symbionts undergo a complex dialogue in order to form a mutual relashionship that firstly involves plant root exudates that induce the synthesis of the Nod factors in bacteria. In the root hair cells follow the calcium signalling, the root hair deformation, a complex signaling cascade initiated by the calcium oscillations, resulting in expression of nitrogen fixation proteins and division of cortical cells in order to form an anatomical structure, the nodule, and only after certain steps are crossed, following stages will set off. The bacteria experience massive changes in order to specialise for the nitrogen fixation, at the level of DNA expression, structure, transmembrane transportation and biochemistry.

Keywords: symbiosis, Nod factor, calcium signalling.

Introduction

Nitrogen, as we know, is found in the atmosphere in a great proportion (79%), though it cannot be assimilated by superior organisms because of the triple bond in the molecule (N≡N). It is, also, indispensable for life to develop, contributing to the bricks of it: amino acids and nucleic acids. The only organisms capable of transforming the inaccessible molecule into an easily assimilated one (ammonia) are the diazotrophic bacteria, free-living or symbiotic. The *Rhizobium* genus is well-known for its ability to form symbioses with plants from the *Fabaceae* family, relationships with beneficial effects for the entire agricultural system, since nitrogen rich plants, besides having an enhanced production, also fertilise the soil after death. The bacteria therefore supply the plant with utilizable nitrogen, receiving in exchange carbohydrates, accommodation in a suitable organ – the nodule, and optimal conditions for the reduction of nitrogen. With all the benefits, this liaison is not mandatory, both organisms being capable of autonomous survival, each new plant generation requiring population by the free bacteria in order for the symbiosis to be established. But for such a mutualism to be instituted a tremendous message exchange between the two parts is necessitated (Gitig, 2010; Kneip et al., 2007; Lee and Hirsch, 2006).

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First step: communicating from a distance

Plants are continuously secreting the secondary metabolites flavonoids in their rhizosphere via ABC transporters, or the MATE proteins (multidrug and toxic compound extrusion) but the amount eliminated grows then in vicinity are representatives of the compatible *Rhizobium* species. A great specificity can be noticed at this level, as certain bacterial species are attracted by just a few flavonoids, and different legume species will only secrete certain ranges of chemo-attractants. One specific flavonoid can be a chemo-attractant for the paired bacteria, while it is repellent for some other species (Cesco et al., 2010). Yet certain plant species can form symbioses with several bacterial species if the palette of secreted flavonoids corresponds. Therefore flavonoids will bind a bacterial protein that acts as a transcription activator, the NodD protein (Broughton et al., 2000). It, once activated, will lead to the transcription of the *nod* genes: *nodD*, with its reglatory function and *nodABC*, which encode enzymes for the basic frame of the Nod Factor (NF). Other *nod* genes products serve for adding different side-chains, such as fucosyl, sulphuryl, acetyl, methyl, carbamoyl and arabinosyl residues to the backbone of the NF, which is a β-1,4-N-acetyl-glucosamine (chitin) oligomer of three to five units, with an acyl chain of 16-20 carbons attached at the non-reducing end (Jones et al., 2007; Lee and Hirsch, 2006; Streng et al., 2011).

Recent studies done with the help of *aequorin* (a luminescent protein that reacts to the Ca^{2+} concentration in the cell) showed that not only in the plant cell appears calcium signalling in the process of settling the symbiosis, but also in the bacterial cells: right after the contact with the compatible plant roots, in rhizobia appears a sudden increase in the Ca^{2+} concentration, and another peak after 10 to 15 minutes, and only after this the *nodABC* genes begin transcription. Also, if EGTA (ethylene glycol tetraacetic acid) is added, the event is missed, showing the extracellular origin of the calcium. Another proof of the high specificity of the symbioses lies in the extent to which the calcium response forms, with higher intensity in the case of compatible "couples" and lower in unaccustomed pairs, with an even poorer effect in reaction to non-legumes (Moscatiello et al., 2009).

First effects in the plant

A few minutes after the attachment of the rhizobia to the root hair cell and the secretion of the NF, an increase in the calcium concentration appears, therefore a depolarisation of the membrane, by influx of Ca ions from the outside and probably by elimination from intracytoplasmic stocks. The increase in calcium concentration begins at the apex of the root hair and travels towards the base, this being considered the probable cause of the root hair deformation (Lee and Hirsch, 2006; Shaw and Long, 2003). If in the medium is added EGTA the depolarisation of the membrane ceases to appear, along with the loss of induction of the nodulation genes. After the calcium influx, event observed

along the entire root hair cell, lasting for 1 to 3 minutes, the elevated Ca^{2+} levels are maintained until the calcium oscillations appear. They are manifested especially in the cytoplasmic area around the nucleus after a lag of 7-25 minutes and can last for an entire hour. These effects were obtained from addition of 10 nM NF to the medium (Shaw and Long, 2003).

The root hair deformation is the following step in the evolution of the symbiosis formation and it is considered that the calcium oscillations are the cause. The actin microfilaments in the cell, previously parallel, will now break and accumulate at the tip, and after a few minutes an unordered repolymerisation will begin, resulting in the swelling, bifurcation or curving of the root hair, but mostly in a bending of 360°, forming the so-called "shepperd`s crook" and capturing inside the hook a few bacterial cells. By adding cytochalasin, an inhibitor of actin polymerisation, these alterations do not appear, thus proving the responsible element is indeed, the actin (Lee and Hirsch, 2006).

The signalling pathway induced by the Nod factor

The NF released from the rhizobia will also activate a signalling cascade in the root hair cell, leading to transcription of nodulation genes and will induce cell divisions in the corresponding cortical area of the root (Broughton et al., 2000; Jones et al., 2007; Lee and Hirsch, 2006).

Considering the picomolar concentration of the NF required for the plant to feel, a high affinity receptor must exist. For each of the studied legumes were found groups of two transmembrane proteins containing lysine motifs (LysM). These LysM receptors are specialised for the reception of chitin, explaining their function in apprehending the lipo-chitooligosaccharidic Nod factor. The first two proteins in this signalling cascade are named according to their provenance legume: MtNFP (NF Perception) and MtLYK3/HCL from *Medicago truncatula*; LjNFR1 and LjNFR5 (NF Receptor) from *Lotus japonicus*; and PsSYM10 and PsSYM2A from *Pisum sativum* (Jones et al., 2007; Lee and Hirsch, 2006). At least in the case of LjNFR1 and LjNFR5, using a two-phase partitioning system, the cellular localisation was established to be the plasma membrane. The two perform their function as heterodimers. This association is essential considering the fact that the kinasic activity of the LjNFR5 is missing and the signal triggered by the perception of the NF can only be further transmitted in association with LjNFR1 (Madsen et al., 2011; Nakagawa et al., 2011). The amino acid sequence of the LjNFR1 is almost identical to that of AtCERK1, a chitin receptor involved in PTI (PAMP-triggered immunity), with only a few substitutions in the kinasic domain. For this reason, it is considered that the NF receptors have evolved from pattern recognition receptors (PRRs) (Nakagawa et al., 2011).

Further, a Leucine-rich regions protein (LRR) with a Serine/Threonine-Kinase region interacts with the other proteins of the cascade, transmitting the signal by its kinase function. Again, naming coincides with the plant species: MtDMI2 (Doesn't Make Infection); PsSYM19; LjSYMRK (Symbiosis Receptor-like Kinase); MsNORK (Nodule Receptor-like Kinase) from *Medicago sativa* (Jones et al., 2007; Lee and Hirsch, 2006).

Next, ion channels containing Leucine-zipper domains actuate. Identified forms are MtDMI1 and the twin proteins CASTOR and POLLUX (Lj). Homologous genes of these twin proteins appear in all plant types (*Vitis, Zea, Oryza, Populus* etc.), but in *Arabidopsis* was noticed an exception, the absence of the CASTOR protein. This deletion would give an explanation to why the *Brassicaceaea* family does not form symbiosis (Chen et al., 2009). CASTOR and POLLUX are nonspecific ion channels with permeability for both Ca^{2+} and K^{+}. Both proteins contain 1, respectively 3 nuclear localisation signals. CASTOR seems to be localised in the outer nuclear envelope, and POLLUX in both envelopes (Charpentier et al., 2008).

A Ca^{2+}/Calmodulin-dependent protein-Kinase-like (CCaMK), containing three EF hands motifs, calcium binding motifs follows. This protein senses nanomolar differences in Ca^{2+} concentration, values comparable with the ones entering the cell as answer to the NF. Plants that are mutant for this gene present a Ca^{2+} influx but not an activation of expression of nodulation genes, placing the protein downstream of the calcium influx, but upstream of the gene activation, probably it is exactly the element correlation the Ca^{2+} concentration variation, as a secundary messenger, with nodulation genes induction (Mitra et al., 2004). Representatives are: MtDMI3, PsSYM9. These kinases have nuclear localisation, and this is where they interact with the CYCLOPS protein (*Lotus japonicus*), further controlling the nodule development (Yano et al., 2008).

The kinasic action of the last proteins activates the transcriptional regulatory factors belonging to the GRAS family: MtNSP1 and MtNSP2 (Nodulation Signal Pathway). They are supposed to influence the expression level, leading to transcriptional changes (Lee and Hirsch, 2006).

Finally, the transmembrane transcriptional factor LjNIN, MtNIN (Nodule Inception), PsSYM35 is the protein crossing the nuclear envelope, activating the nodulation genes expression, and inducing mitosis in cortical cells, leading to the formation of the nodule primordia (Jones et al., 2007; Lee and Hirsch, 2006). NIN leads to the transcription of the genes encoding LjNF-YA1 and LjNF-YB1, that conjuctively form the CCAAT-box binding NF-Y complex. NIN is considered a mediator between the nodulation signals and the mechanisms associated with cell prolifferation (Soyano et al., 2013).

Another transcription factor discovered is an ERF-type (Ethylene Response Factor), the ERN (ERF required for Nodulation). It is constitutively expressed in the root hair cells, but it`s quantity rises in the presence of the NF. Its function is downstream of the CCaMK, probablly even below NSP and NIN,

being another candidate for the function of DNA binding. But because mutants for this gene are still able to form nodules, it is probably not a vital element, like NSP (Middleton et al., 2007).

Formation of the Infection Thread

Once the "shepperd's crook" is formed, bacteria begin entering the cell through a filiform invagination of the cell membrane called Infection Thread – IT. The growth of the IT is supported by membrane synthesis, more exactly by continuous fusion of Golgi vesicles. This level of symbiosis is also subjected to a great deal of specificity, many unspecific rhizobia-legume trials failing at this step, forming anomalous IT that are aborted or empty, unpopulated nodules (Jones et al., 2007; Robertson et al., 1978).

Bacteria participate to a lesser extent to the formation of the IT than the plant, but mutation in the genes coding enzymes involved in the synthesis of any of the substances needed at this step leads to the formation of unfunctional nodules. Characteristic substances from the rhizobia wall are: cyclic β-glucans, succinoglycans, galactoglucans, exopolysaccharides (EPS), exooligosaccharides (EOS), lipopolysaccharides (LPS) and some secreted proteins. Many genes are involved in the synthesis of these, for example, for the synthesis of the EPS and EOS are known the *exo* genes, coding for enzymes that synthetise the precursors (ExoC, ExoB, ExoN, ExoY), or enzymes participating in the elongation of the molecule back-bone (ExoA, ExoL, ExoM, ExoO, ExoW). ExoZ, ExoH, ExoV add different side-chains to the back-bone, ExoP, ExoQ, ExoT, and ExoF transport the monosaccharides to the main chain on the outer side of the cell, and ExoK and ExoH cut the EPS into EOS of 8-9 monomers, giving specificity (Broughton et al., 2000; Jones et al., 2007; Staehelin et al., 2006).

The plant closely controls the IT formation with the complexity and abundence of the substances in the root hair cell participating at this phase: arabinogalactans, xyloglucans, esterified and non-esterified pectin, cellulose, ATPase, lipoxygenases, and other proteins (Broughton et al., 2000). It was found that proteins from the NF induced signalling cascade are important in the correct formation of the IT, as mutants with a reduced expression of the MtNFP and MtLYK3/HCL formed aberrant IT, with a normal cortical cell mitosis (Jones et al., 2007). Also, flotilin proteins, especially FLOT4 seem to be important in the growth of the IT, but not in the growth and curling of the root hair. Animal flotilins are involved in membrane modelling processes, actin binding, as markers of lipid rafts, in endocytosis, in filopodia formation, so its implication in IT formation is understandable. Induction of FLOT4 expression does not appear after contact with pure NF, LPS, cyclic β-glucans or succinoglycans, but only after the entire bacteria is present, so far the exact substance producing this effect being still unknown (Haney and Long, 2010).

The penetration of the plant cells by the rhizobia

The elongation of the IT leads the rhizobia to the cortical area where the nodule primordium has formed. These cells have stopped dividing and are polyploid in order to be able to handle the high metabolic rate needed for nitrogen fixation. There are two types of nodules, oblong, undetermined ones, found in most of the legumes from the temperate climate, characterised by a leveling of the nodule in: the meristematic area, in the apex of the nodule, composed of dividing cortical cells amid which the IT has passed; beneath this – zone II, with cells and bacteria in an ongoing differentiation, zone III, where the nitrogen fixation happens, and zone IV, the senescent zone. The other type of nodules, the determined ones, are heterogenous, spherical, and are characteristic for tropical legumes (*Glycine max, Arachis hypogaea*) (Jones et al., 2007).

When the IT reaches the nodule primordia, the bacteria are endocytosed by the plant cells, forming the symbiosomes, rhizobia surrounded by a peribacteroid membrane. The bacteria enter the cells in the area where the cell wall has collapsed as a result of sudden growth or when the IT penetrates the cell wall. In undetermined nodules, the bacteria and the surrounding membrane will multiply, radially filling a large proportion of the cell, which will undergo a rearrangement of the cytoscheleton and organelles, while in the detemined nodules, inside one peribacteroid membrane the bacteria will divide. The demand for membrane biogenesis is 30 times greater in an infected cell compared to a non-infected one (Brewin, 2004; Ivanov et al., 2012; Limpens et al., 2005).

Freeing of the bacteria from the IT is a process controlled by both parties, a plant deficient in certain factors might entrap the rhizobia in the IT or it might over-extend the IT, while the bacteria might not come out of the IT. An example is the DMI2 deficiency, when the IT interminably grows, leading to a phenotype characteristic to primitive legumes, where the bacteria remain captive in the IT, thus making it a "fixation thread" (Limpens et al., 2005).

Once the rhizobia have been freed and successfully endocytosed, many lipids and proteins are targeted from the plant towards the symbiosomes, or are now syntetised by the bacterium, leading to the formation, processing and maturation of a distinct biochemical compartiment (Jones et al., 2007).

Differentiation of the bacteroids

The bacteria meeting the requirments for nitrogen fixation will become bacteroids, with new morphological and biochemical properties. The rhizobia elongate, and the genes for most of the metabolic pathways are inhibited, with the sole stimulation of the expression of the nitrogen fixation and respiration genes. N_2 fixation is a costly process, using 16-18 moles of ATP/ mole of N_2, demanding for an augmented respiration. Being so energy consuming, nitrogen

fixation will never occur in a soil with enough nitrates, being triggered by a lack of nitrogen in the rhizosphere (White et al., 2007).

Only after the symbiosomes have filled the volume of the infected cells and the plant feels a decrease in the oxygen concentration through the FixL-FixJ-oxygen sensitive regulatory system, that the nitrogenase complex encoded by the *nif* (nitrogen fixation) genes will be synthesised (Gibson et al., 2008). The peribacteroid membrane acts as a diffusion barrier for the O_2, leading to concentrations of 3-22 nM of free oxygen. Also, leghemoglobin, produced partly by the plant – the globin part, and partly by the bacteria – the hem, strongly binds excess oxygen, and leaves it only for the respiration of the bacteroids. All these efforts are done because the nitrogenase is sensitive to O_2 and an increase in its concentration will decrease the enzyme yield. LegHb represents 25% of all the soluble proteins in a nodule, making the functional nodules pink (Gibson et al., 2008; Lee and Hirsch, 2006).

The genetic material of the bacteroids also suffers an endoreduplication in order to increase productivity. For the intense DNA synthesis, bacteria have dNTPs synthesising enzymes and transport systems in the peribacteroid membrane for phosphate import (Jones et al., 2007).

Deffinitivation of symbiosis

The membranes of the symbiosome have well shipshaped transport systems for the necessary exchange with the plant cell. The plant ensures the appropriate amount of O_2 for respiration and offers energy sources partially prepaired (malate, sucrose), receiving instead NH_4^+, that is transported to the plant via the amino acids Glutamine-Glutamate, Asparagine-Aspartate (Gibson et al., 2008), but also via Alanine, by diffusion in the membranes, or by Mg^{2+} or Ca^{2}-dependent antiport channels. If the Gln-Glu system is inhibited, the plants will be chlorotic (White et al., 2007).

Other necessary transporters are specific for sulphates and iron, elements constituting the Fe-S cluster of the nitrogenase enzyme (Jones et al., 2007; White et al., 2007).

In determined nodules some cells are not infected, and are specialised in taking over and further assimilation of NH_4^+ into ureides and amides, that will subsequenly be delivered to the rest of the plant (Gibson et al., 2008).

Conclusions

This paper briefly presents the steps in the establishment of the symbiotic relationship between diazotroph, nitrogen fixing bacteria and plants from the *Fabaceae* family.

Much is still unknown about the immense communication between symbionts, much is just partially understood, and some information might be

even speculated and not demonstrated, so a lot more work is to be done in order to fully understand the process.

Rezumat

Simbionţii participă la un dialog complex pentru a forma o relaţie de mutualism, dialog ce presupune în primă fază emiterea unor substanţe de către rădăcini şi sinteza factorilor Nod ca răspuns al bacteriilor faţă de acestea. În celulele perişorului absorbant urmează semnalizarea prin calciu, deformarea perişorului, declanşarea cascadelor de semnalizare iniţiate de oscilaţii de calciu. Acestea duc la expresia proteinelor de fixare a azotului şi la diviziunea celulelor corticale ce vor forma noua structură anatomică, nodozitatea. Bacteriile suferă schimbări masive pentru a se specializa pentru fixarea azotului la nivelul expresiei genice, al structurii, al transportului transmembranar şi al întregii biochimii.

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BUCHBESPRECHUNG

WERTVOLLE MONOGRAPHIE DES RODNAER – GEBIRGES ERSCHIENEN

Rudolf RÖSLER - Regensburg

Die Verwaltung des Nationalparks Rodnaer–Gebirge, veröffentlichte 2011 unter Federführung von Dr. **Claudiu Iuşan** eine vielseitige und wertvolle Monographie dieses Bergmassivs unter dem Titel: **Monographie des Nationalparks Rodnaer – Gebirge (Biosphärenreservat),** Originaltitel: **Monografia Parcului Naţional Munţii Rodnei (Rezervaţie a Biosferei),** erschienen 2011 im Verlag Editura Todesco, Cluj - Napoca, ISBN 978-606-595-011-5, 335 Seiten, mit zwei Abbildungen und 38 Tabellen.

Dem Herausgeber ist es gelungen, die zahlreichen Forschungsergebnisse der letzten Jahre aus diesem Großraum, durchgeführt von namhaften rumänischen und mitteleuropäischen Fachleuten, zu einer Einheit zusammenzuschweißen. Nach einer Einführung über Entstehen (1990) und Beschreibung des Nationalparks (Fläche = 46.417, 1 ha), werden die Kapitel Geologie, Geomorphologie, Hydrologie sowie die Böden dieses Bergmassivs behandelt. Es folgt der Abschnitt Flora und Vegetation, dem auch eine kurze Geschichte der botanischen Erschließung dieser Bergwelt beigegeben ist.

Die Flora wird wie folgt gegliedert:

Pilzflora, vertreten durch 390 *Myxomycetes* – Arten (Schleimpilze), von denen vier neue Spezies entdeckt und beschrieben wurden, wie: *Melanconium asperulum* Moesz., *Pestalotia truncata* Lev., *Leptosphaeria glycariae-plicatae* Săvul. & Sandu, sowie *Stresseria rhododendri*. Die *Macromycetes* (Echte Pilze) sind durch 172 Arten vertreten, von denen nennenswert u.a. sind: *Mitrula paludosa* Fr., *Amanita subalpina* L., *Pulveroboletus lignicola* Kallenbach & Pilat u.a.

Flechten (Lichenes) wurden in einer Anzahl von 274 Arten bestimmt, darunter 6 Endemismen, wie: *Thelidium gibbosum* Zsch., *Amorphoblastia rodnensis* Zsch. & Serv., *Verrucaria marmorosica* Serv. u.a.

Moospflanzen (*Bryophyta*) wurden 415 Arten gefunden, darunter zahlreiche Seltenheiten für die Flora Rumäniens, wie: *Bucegia romanica* Radian, *Moerckia blyttii* Moerch & Brockm.., *Anthelia julacea* L. u.a.m.

Kormophytenflora (Großpflanzen): Bisher konnten 1.585 Arten festgestellt werden, davon zahlreiche Endemiten, wie: *Silene nivalis* (Kit.) Rohrb., *Saussurea porcii* Degen u.a.m. Von den zahlreich geschützten Pflanzen erwähnen wir u.a.:

Leontopodium alpinum Cass., *Gentiana lutea* L., *Nigritella rubra* (Wettst.) K. Richt., *Pinus cembra* L. etc.

Die Vegetation ist reichhaltig und in 74 Gesellschaften anzutreffen, von denen 22 erstmals für die Wissenschaft beschrieben wurden. Hier kommen folgende Vegetationsstufen (Höhenstufen) vor: a.) Waldstufe – zwischen 500 und 1.500 m; b.) Subalpine Stufe – 1.500-2.100 m; und c.) Alpine Stufe – 2.100 – 2.300 m. Die flächenreiche Waldstufe wird eingeteilt in: Unterstufe (U.S.) Buchen – Traubeneichen-Wälder (500 - 650 m); U.S. der Buchen-Wälder (650 – 1.100 m) und die U.S. der Buchen – Nadelholz-Wälder (1.100 – 1.500 m). Die Walsdtufe ist als die artenreichste und artenvielfältigste anzusehen.

Wenn Flora und Vegetation des Rodnaer – Gebirges bisher annähernd exhaustiv erforscht wurden, ist der Beitrag zur

Fauna in besonderem Maße zu begrüßen. Rund 3.000 Arten wurden bestimmt, dabei zahlreiche Endemiten dieses Gebirgstockes entdeckt, wie: *Romanosoma cavernicola*, *R. bîrtei*, *R. odici* u.a.m. Die durchgeführten Inventuren ergaben folgende Daten: *Enchyträen* (Wenigborster) = 40 Arten; *Lumbricidae* (Regenwürmer) = 27 Arten; *Nematoden* (Fadenwürmer) = 75 Arten; *Collembolen* (Springschwänze) = 115 Arten (so auch *Tetrachanthella transylvanica*); *Diplopoden* (Doppelfüßer) = 43 Arten (so z.B. *Polydesmus tetramus* rodnaensis); *Chilopoden* (Hundertfüßer) = 49 Arten (so *Clinopodus* rodnaensis); *Amoebozoa* = 30 Arten; *Acari* (Milben) = 201 Arten; *Araneae* (Spinnen) = 115 Arten; *Diptera* (Zweiflügler) = 245 Arten; *Ephemeriden* (Eintagsfliegen) = 49 Arten; *Heteroptera* (Wanzen) = 164 Arten; Hymenoptera (Hautflügler) = 62 Arten; *Homoptera* (Gleichflügler) = 115 Arten; *Isopoda* (Asseln) = 9 Arten; *Coleoptera* (Käfer) = 1.035 Arten (darunter *Bembidion transsylvanicus* und *Carabus zawadskii*); *Lepidoptera* (Schmetterlinge) = 642 Arten (so auch eine neue Art für Rumänien – *Apotomis infida*); *Orthopteren* (Geradeflügler) = 52 Arten (so *Pholidoptera transsylvanica*); *Odonata* (Libellen) = 40 Arten; *Opiliones* (Weberknechte) = 31 Arten; *Plecoptera* (Steinfliegen) = 55 Arten; *Psocoptera* (Staubläuse) = 22 Arten; *Trichoptera* (Köcherfliegen) = 34 Arten; Muscheln = 78 Arten; *Crustacea* (Krebse) = 38 Arten; *Rotatoria* (Rädertiere) = 49 Arten; Fische = 28 Arten; *Amphibien* (Lurche) und *Reptilien* (Kriechtiere) = 15 bzw. 10 Arten; Vögel = 157 Arten; Säugetiere = 54 Arten.

Dem Werk ist eine umfangreiche Literaturliste (409 Titel) beigegeben, sowie eine Karte des Nationalparks. Es sei zu erwähnen, dass dieses Buch wertvolle Unterlagen liefert nicht nur für Biologen (Botaniker und Zoologen), sondern auch für die Wirtschaftszweige Forstwesen, Landwirtschaft, Tierzucht etc.

Recenzie:
MONOGRAFIA PARCULUI NAŢIONAL MUNŢII RODNEI, 2011.

Coordonatorul ştiinţific Dr. C. Iuşan a reuşit în mod exemplar să valorifice rezultatele cercetărilor multilaterale, efectuate în ultimii ani în Munţii Rodnei. După o scurtă introducere (înfiinţarea parcului în anul 1990, managementul etc.) se trece la o descriere a acestuia (46.417,1 ha) după următoarele capitole: geologia, geomorfologia, hidrologia, clima, solurile, flora şi vegetaţia, precum şi fauna Munţilor Rodnei. Pentru o mai uşoară folosire a marii diversităţi a datelor de către specialiştii din străinătate, flora şi fauna sunt tratate după mari grupe sistematice de plante (ca de exemplu: ciuperci, licheni, cormofite) şi animale (încrengături), amintindu-se şi numărul de specii identificate în Parcul Naţional Munţii Rodnei. În unele cazuri sunt date ca exemplu speciile cu deosebită importanţă sistematică, endemică sau descoperite în ultimii ani. Această carte este valoroasă nu numai pentru naturalişti (botanişti, zoologi etc.), ci şi pentru practicienii din producţie (silvicultori, agricultori, zootehnişti etc.). Un număr de 409 titluri consultate încheie lucrarea, dând astfel cercetătorilor interesaţi posibilitatea de a se documenta amănunţit în vederea studierii multiplelor tematici prezentate.

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