Acta Universitatis Sapientiae

Agriculture and Environment

Volume 5, 2013

Sapientia Hungarian University of Transylvania
Scientia Publishing House

Contents

Acta Universitatis Sapientiae
Agriculture and Environment, **5**, (2013) 5–18

Effects of cycocel priming on growth and early development of rapeseed under drought stress

Alireza POURMOHAMMAD
email: pourmohammad@ymail.com

Fariborz SHEKARI
email: fb_shekari@yahoo.com

Veeda SOLTANIBAND
email: soltani.agri@gmail.com

University of Maragheh, Faculty of Agriculture,
Department of Agronomy and Plant Breeding,
Maragheh, Iran

Abstract. An experiment was concluded to evaluate the effects of priming on germination characteristics and establishment of rapeseed seedlings during three stages. In the first and second stages of experiment, the effects at three factors vis: cultivars (RVS, Hayola), water-deficit stress (Control,-6 and -12 bar) and cycocel treatment (dry seeds, 300, 600, 900, 1200 and 1500 μM) on germination and seedling growth of plant were assayed. The results showed that priming had positive effects on germination percentage, germination rate index, germination index, and radicle length under water-deficit conditions. RVS had greater efficiency than other cultivars. The third section of experiment revealed that the priming of RVS seeds improved the stem, root and dry weight, total dry weight as well as leaf number and area of seedlings.

Keywords: germination, seedling growth and establishment, LAR, LWR

1 Introduction

Germination is the first stage of plant development, having crucial role in seedling emergence and establishment. Germination is greatly affected by environmental stimuli, especially temperature and soil humidity [3]. Since seedling establishment is a sensitive stage in crop growth and development with direct planting, uniformity and high emergence percentage have prominent effects on yield and production quality [30].

Rapeseed, an important oil-bearing crop, has been shown to be adapted in water-deficit conditions [1]. Owing to the prevalence of semi-dry growing locations in most parts of Iran, rapeseed may be a promising crop that meets the demands for edible oil [1, 12]. Since the main production of rapeseed occurs under dry-land farming, plant responses to drought stress are worthy of great consideration [13]. Optimum seed germination and emergence potential under drought conditions provide the better establishment, and subsequently lead to yield increment [5].

Recently, huge attention has been paid to the enhancement of seed vigour for greater germination and seedling growth parameter under extreme environments [21]. Priming has been defined as a useful method for the improvement in seed germination and subsequent growth under stressful conditions [13]. Several studies have been demonstrated the prominent positive effects of priming on germination-related traits of several crop species [22, 23, 28]. In general, priming affects the hydration / dehydration ratio of seeds in favour of increased and hastened germination percentage and rate [8]. This in part promotes the seedling establishment and its later growth and development [17]. From biochemical and physiological point of view, priming goes to increases in antioxidant pool [14], the amount and translocation of soluble sugars [19] and protein biosynthesis [10]. Also, priming enhances the transcription and translocation of some genes [11] and affects membrane integrity [6]. CCC (Cycocel) is a widely employed growth retardant composed of a four-member oniumic group [2]. Scarisbrick et al. [25] noted that cycocel effects on plant growth and development were highly dependent on cultivar and species. Drought tolerance has been defined as the most common consequence of exogenous application of cycocel on most plant species [25].

In the present experiment, we aim to evaluate the effects of CCC on germination and early growth of several rapeseed cultivars under water-deficit conditions.

2 Materials and methods

This experiment was conducted in the Research Laboratory of the Agronomy and Plant Breeding Department at the University of Maragheh during autumn 2009. The treatments were two spring rapeseed cultivars (Hayola 401 and RVS), drought stress levels using sucrose (0, -6 and -12 bar) and cycocel concentrations (0, 300, 600, 900, 1200 and 1500 μM). Experimental design was factorial based on CRD with three replications. The seeds were surface-sterilized with 5% sodium hypochlorite. Cycocel treatments were applied on seeds for 24 hrs at 10°C. Then seeds were rinsed with sterile distilled water and were left to dry till they reached their primary weight. In the first step, 50 seeds placed in a Petri dish between filter papers were transferred into incubator at 20°C for 7 days. The seeds were surveyed daily and FGP (Final Germination Percentage), CVG (Coefficient for Velocity of Germination), GRI (Germination Rate Index) and GI (Germination Index) were measured [15]. In the second series of experiments, the primed seeds wrapped in filter papers were transferred into incubator at 20°C for 7 days. The drought stress treatment was the same as the first experiments. The recorded traits beyond seven days were radicle length, plumule length, plumule dry weight, radicle dry weight and dry seed weight. In the third experiment, primed and control seeds (RVS cultivar) were planted in the pots at a depth of 15 mm and maintained at 20±2°C and 16:8 photoperiod. Experimental design was RCB with 4 replications. Drought stress levels were measured using control plants with 100% water availability and 30, 50 and 70 % of available water computed by:

$$\begin{aligned}
&\mathrm{L} = (1 - \mathrm{PAW_t}/\mathrm{AWC}) \times 100\\
&\mathrm{PAW_t} = \mathrm{SMC} - \mathrm{WP}\\
&\mathrm{AWC} = \mathrm{FC} - \mathrm{WP},
\end{aligned}$$

where $\mathrm{PAW_t}$ is plant available water in defined time (based on a 24-h period), AWC is available water content between wilting point (WP) and field capacity (FC), and SMC is soil moisture content in defined time (based on a 24-h period). Wilting point was quantified by pressure-plate system, and the treatments were applied based on weight percentage. At the end of the 40-day period, emerged seeds percentage, stem dry weight, leaf dry weight, total dry weight, leaf number, leaf area ratio (LAR), stem/root ratio (S/R), and leaf weight ratio were measured. Data were analysed with SAS and MSTATC software, and graphs were drawn in Excel programme. Mean comparisons were carried out by Duncan's multiple range test at $P < 0.01$.

3 Results and discussions

In the first stage of the experiment, CCC improved all growth-related traits. Based on the results of cycocel treatment, stress levels and interaction effects of stress and cultivar were significant on FGP ($P \leq 0.01$). Mean comparison revealed that cycocel application had significant effect on FGP. However, there was no significant difference between cycocel levels (Table 1).

Table 1: The effects of cycocel amounts on rapeseed germination characteristics in the first experiment

	Final Germination Percentage (FGP)	Coefficient for Velocity of Germination (CVG)	Germination Index (GI)
0 μM	70.9 b	0.35 b	73.6 b
300 μM	73.6 ab	0.44 a	84.8 a
600 μM	79.7 a	0.48 a	90.0 a
900 μM	80.7 a	0.45 a	87.8 a
1200 μM	80.3 a	0.42 a	92.7 a
1500 μM	81.1 a	0.45 a	91.7 a
Probability Level	**	**	**

** Means significant at P$\leq$ 0.01 based on Duncan's multiple range test

Under normal (non-stress) conditions, FGP values were non-significant for all the studied varieties; however, with severe stress, RVS demonstrated the higher FGP values compared to other cultivars (Table 3). CVG values in case of all pretreated seeds were higher than dry seeds (Table 2).

Table 2: The effects of drought stress levels on rapeseed germination and seedling characteristics in the first and second experiment, respectively

	Coefficient for Velocity of Germination	Plumule dry weight (g)	Radicle dry weight (g)	Plumule length (cm)
0 bar	0.68 a	0.0130 a	0.008 a	3.10 a
-6 bar	0.40 b	0.0120 a	0.004 b	0.89 b
-12 bar	0.22 c	0.0001 b	0.001 c	0.04 c
Probability Level	**	**	**	

** Means significant at P$\leq$ 0.01 based on Duncan's multiple range test

Table 3: The interaction of drought stress levels and rapeseed cultivars on germination and seedling characteristics in the first and second experiment

	Final Germination Percentage (FGP)	Germination Rate Index (GI)	Germination Index (GI)	Seed dry weight
RVS× 0 bar	90.83 a	71.52 a	119.3 a	0.1556 e
RVS× -6 bar	93.61 a	74.83 a	122.1 a	0.2333 d
RVS× -12 bar	86.94 a	37.38 c	95.8 c	0.8389 a
Hayola 401× 0 bar	93.33 a	44.27 b	108.2 b	0.3889 c
Hayola 401× -6 bar	31.94 c	6.690 e	21.5 e	0.4944 b
Hayola 401× -12 bar	68.89 b	19.10 d	53.6 d	0.0085 a
Probability Level	**	**	**	**

** Means significant at P≤ 0.01 based on Duncan's multiple range test

In contrast, severe drought stress led to reduced amounts of CVG (Table 1). Homogenous and increased germination percentage behind cycocel pretreatment has been demonstrated earlier [4]. For instance, the priming of corn seeds with polyethylene glycol 4000, urea and water had promising effects on germination rate and percentage [21]. These results are similar to the findings of Shad et al. [26] in soybean. Also, Shekari [27] reported that salt and some other ionic solution pretreatments enhanced the germination-related traits in rapeseed. Emergence of pepper plants was also positively influenced by CCC pretreatment in Demir & Ellis [9] research. Increase in respiration potential and ATP generation, as well as enhanced transcription levels and protein synthesis have been reported as the main effects of the seeds pretreatment with priming agents [9]. Rashid et al. [24] reported that hydropriming led to early germination, higher establishment and increased yield in some crops under normal and stressful growing conditions. Early emergence of seedlings in regions with limited growth period is worthy of great attention. In other words, early and hastened leaf area expansion goes to the optimum light perception and subsequently greater growth potential compared with weeds. These all finally lead to increased dry matter accumulation and yield [1].

The results also showed that the main and interaction effects of cycocel*stress and stress*cultivar were significant. Cycocel treatment with non-stress conditions had higher values for most traits than dry seeds (Table 4). With the increase in water-deficit levels and at -6 bars, primed seeds treated with 600 μM cycocel had higher GRI values than control seeds, but the difference within cycocel levels was not significant.

Table 4: The interaction of drought stress levels and cycocel amounts on germination and seedling characteristics in the first and second experiment

Treatment	Germination Rate Index (GRI)	Seed dry weight	Treatment	Germination Rate Index (GRI)	Seed dry weight
Non-treatment×0 bar	52.6 b	0.0037 defg	900 $\mu m\times$ 0 bar	72.29 a	0.0038 cdefg
Non-treatment× -6 bar	35.5 d	0.0043 cdefg	900 $\mu m\times$ -6 bar	46.02 cd	0.0102 ab
Non-treatment× -12 bar	10.3 e	0.0005 fg	900 $\mu m\times$ -12 bar	15.21 e	0.0050 cde
300 $\mu M\times$ 0 bar	76.4 a	0.0053 cde	1200 $\mu m\times$ 0 bar	78.64 a	0.0037 defg
300 $\mu M\times$ -6 bar	37.8 cd	0.0080 abc	1200 $\mu m\times$ -6 bar	40.02 cd	0.0098 ab
300 $\mu M\times$ -12 bar	9.4 e	0.0003 g	1200 $\mu m\times$ -12 bar	13.13 e	0.0032 defg
600 $\mu M\times$ 0 bar	82.1 a	0.0053cde	1500 $\mu m\times$ 0 bar	77.94 a	0.0047 cdefg
600 $\mu M\times$ -6 bar	45.5 bc	0.0118 a	1500 $\mu m\times$ -6 bar	44.09 bc	0.0065 bcd
600 $\mu M\times$ -12 bar	13.5 e	0.0015 efg	1500 $\mu m\times$ -12 bar	15.86 d	0.0012 efg

** Means significant at $P\leq 0.01$ based on Duncan's multiple range test

Under severe stress conditions (-12 bar), there was no significant difference between primed seeds and control ones (Table 4). Mean comparison revealed that the studied verities had no significant difference under non-stress conditions, but under stress conditions – under both mild and intense stress levels–, 'RVS' had the higher GRI amount compared to other cultivars (Table 3). The main and interaction effects of stress*cultivar were significant ($P \leq 0.01$) on GRI. GRI ratios for primed seeds were higher than those for non-primed seeds, whereas dry seeds had the lowest GRI values (Table 1). Under normal conditions, there was no difference among cultivars regarding GRI, but under intense stress, RVS was more promising than other cultivars, mainly due to its greater efficiency for early growth and development, especially under stressful conditions. Moradi Dezfuli et al. [21] reported the same results. Shekari et al. [27] also reported that the different priming solutions also increased the GRI values for rapeseed.

In the second part of the experiment, cycocel pretreatment increased the radicle length. In this case, the main and the interaction effects of pretreatment*stress levels as well as the interaction of pretreatment stress levels*cultivar were significant on radicle length (Table 5). Mean comparisons revealed that, under non-stress conditions, primed seeds disposed of higher radicle length compared to dry seeds. With mild stress application, primed seeds in both varieties had the highest radical length in contrast with non-treated seeds. In other words, dry seeds had an especially low efficiency for radicle growth and subsequent development (Table 5).

The results from the second section of the experiment revealed that cycocel pretreatment positively influenced the radicle length (Table 5). The interaction effects were significant for the treatments. Considering this, and under non- stress environment, pretreated varieties had the longer radicles compared to control ones. The same trend was observed under mild stressful conditions. In contrast, with severe stress, there were no significant differences between treatments (Table 5). In germination studies, radicle length assay has been defined as a reliable criterion for the prediction of subsequent seedling growth, and as a logical consequence it influences plant growth and development during early growth encountered with drought environment [1]. Following the pretreatment with different solutions [16, 21], the same results have been reported with diverse plant species for radicle length. At the same time, Subedi & Ma [29] noted that GA3 pretreatment not only had no ameliorative effect on radicle length, but in most treatments it also reduced the radicle length in corn. Cycocel pretreatment had no significant influence on plumule length (table not shown).

Table 5: The interaction of drought stress levels, rapeseed cultivars and cycocel amounts of germination on radicle length in the second experiment

Treatment	Mean	Treatment	Mean	Treatment	Mean
Non-treatment×Hayola 401×0 bar	4.67 bcdef	600μM×0 bar ×Hayola 401	0.72 def	1200μM×0 bar×Hayola 401	3.86 def
Non-treatment×RVS×0 bar	3.19 efg	600μM×0 bar×RVS	2.91 efg	1200μM×0 bar×RVS	3.93 def
Non-treatment×-6 bar×Hayola 401	1.43 ghi	600μM×-6 bar×Hayola 401	5.61 abcd	1200μM×-6 bar×Hayola 401	5.81 abcd
Non-treatment×RVS-6 bar×	3.58 defg	600μM×-6 bar×RVS	6.12 abc	1200μM×-6 bar×RVS	6.64 ab
Non-treatment×-12 bar×Hayola 401	0.00 i	600μM×-12 bar×Hayola 401	0.10 i	1200 μM×-12 bar×Hayola 401	0.15 i
Non-treatment×-12 bar×RVS	0.11 i	600μM×12 bar×RVS	0.82 hi	1200μM×12 bar×RVS	0.49 hi
300μM × 0 bar×Hayola 401	4.97 bcde	900μM×0 bar×Hayola 401	4.33 cdef	1500μM×0 bar×Hayola 401	3.24 efg
300μM×0 bar×RVS	4.04 cdef	900μM×0 bar×RVS	2.98 efg	1500μM×0 bar×RVS	4.73 bcdef
300μM×-6 bar×Hayola 401	6.12 abc	900μM×-6 bar×Hayola 401	4.28 cdef	1500μM×-6 bar×Hayola 401	3.31 efg
300μM×-6 bar×RVS	4.67 bcdef	900μM×-6 bar×RVS	7.28 a	1500μM×-6 bar×RVS	2.46 fgh
300μM×-12 bar×Hayola 401	0.00 i	900 μM×-12 bar×Hayola 401	0.76 hi	1500μM×-12 bar×Hayola 401	0.14 i
300μM×-12 br×RVS	0.00 i	900 μM×-12 bar×RVS	0.19 i	1500μM×-12 bar×RVS	0.04 i
Probability Level	**	Probability Level	**	Probability Level	**

Any increase in the stress intensity led to reduced plumule length. The same results were observed in the case of plumule and radicle dry weight (table not shown). Several previous studies reported that using hormonal and non-hormonal solutions for pretreatment of seeds may have divergent effects on the aforementioned traits [27, 29]. Thus, the effects may be promotive, preventative and, in some cases, natural. These variant effects may be in main part due to the agent type, its amount and the plant species. For seed dry weight, interactions of pretreatment*stress level and stress level*cultivar were significant ($P \leq 0.01$). Accordingly, the highest data for seed dry weight was recorded in the third level of stress (Table 4). For the stress and cultivar interaction, the results showed that under severe stress conditions, the greatest seed dry weight belonged to "Hayola 401". However, there was no meaningful difference between varieties considering the above trait. This all means that in RVS cultivar seed storage compounds may have higher mobility during the germination process and the later potential enhances the seeds' germination capabilities, and subsequently increases the seedling establishment under field-grown conditions [27].

In the third section of the experiment, total dry weight (sum of aerial and underground parts) produced the greatest data with 100% available moisture and 900 μM cycocel (table not shown). The same trend was detectable with any increase in stress intensity. Regarding this trait, 600 μM cycocel treatment possessed the next rank (table not shown). With all stress levels, dry seeds (control) had the least amount for seed dry weight. Like with ours, Shekari et al. [27] reported that salicylic acid treatment increased the total dry weight of *Borago officinalis* L. seedlings. Kaydan et al. [18] also reported that under salinity stress conditions, hormonal pretreatment enhanced the seedling dry weight of wheat. The same results were recorded for aerial parts (leaves and stem) dry weight (table not shown). Root dry weight followed the same pattern as well, so that, the application of 600 and 900 μM cycocel had the most promising effect on this trait (table not shown). Simultaneously, with all stress levels, any shortage in available water content led to the reduced root growth. Under severe stress, the prominent decrease in root growth was evidenced. This is a classical viewpoint that, under severe drought stress, photoassimilate partitioning towards the roots adversely affected, and this in turn goes to reduced root growth rate and diminished underground dry weight [27]. Shekari et al. [27] demonstrated that *Brassica rapa* seeds pretreated with different ionic and non-ionic solutions had increased shoot and root dry weight. Pretreated seeds gained the highest data for leaf number and area compared with control non-treated seeds. The 100 μM cycocel treatment produced the near-maximum data for leaf number and area. Our findings are well in conformity with the reports of Martin-Mex [20] and Shekari et al.[27]. Aerial to underground parts ratio (S/R) was affected by the treatments, and with all pretreated seeds the ratio was lower than with control ones. The data for S/R was aligned with the previous sections of the present experiment. This means that the pretreatments mainly affected the underground parts, and hence the S/R ratio.

Contrarily, leaf area ratio (LAR) and leaf weight ratio (LWR) for pretreated seeds were lower than for control seeds mainly due to elevated total dry weight per leaf area and leaf dry weight (table not shown). This is more highlighted, considering the improved underground parts growth and dry matter accumulation, especially under water-deficit conditions. As previously understood, water deficiency affects the growth potential of plants in favour of the underground parts [7]. The results from the present experiment verify the great effects of cycocel on plant growth and development. Furthermore, the findings emphasize the interaction effects of drought stress, varieties and their crosstalk with hormonal pretreatment. Overall, cycocel pretreatment has the potential to affect the pre-germination stages of seeds in favour of improved early growth and development of the seedlings, particularly under water-deficit stressful conditions. However, its effect is partly species dependent. Finally, in the present experiment, cycocel at 600-1200 μM had promising effect on the seeds' germination-related traits, and RVS had also more potential for stress escape with cycocel pretreatment. The other main advantage of RVS was its strong response to severe stress levels.

4 Conclusion

However, the positive effects of CCC coping with drought stress have been frequently verified, but – due to a vast range of feedbacks – there is a great need for further studying its effects and application method on plant growth and development under harsh conditions. In the present experiment, CCC treatment potentiated to enhance the germination-related traits under both normal and stressful conditions. Moreover, varieties had diverse responses to CCC application. Considering seed vigour characteristics, CCC was able to increase radicle length and reservoir mobilization, but, under intense stress, the ameliorative effects of CCC were decreased. Seed priming with CCC significantly affected the plant growth characteristics, so that, total dry weight and photosynthesis potential of plants were amended by the treatments mainly due to the increased leaf number and area as well as the overall plant growth improvement.

References

[1] Alyari, H., Shekari, F. and Shekari, F. (2000), *Oil seed crops: Agronomy and Physiology.* Amidi Publication, Tabriz (in Persian).

[2] Amarjit, B. (2000), *Plant growth regulators in Agriculture and Horticulture: Their role and commercial uses.* Food Products Research, New York.

[3] Anda, A. and Pinter, L. (1994), Sorghum germination and development as influenced by soil temperature and water contact. *Agronomy Journal* 86, pp. 621–624.

[4] Ashraf, M. and Foolad, M. R. (2005), Pre-sowing seed treatment - a shotgun approach to improve germination growth and crop yield under saline and non-saline conditions. *Advances in Agronomy* 88, pp. 223–271.

[5] Baalbaki, R. Z., Zurayk , R. A., Blelk, M. M. and Tahouk, S. N. (1999), Germination and seedling development of drought tolerant and susceptible wheat under moisture stress. *Seed Science and Technology* 27, pp. 291–302.

[6] Bradford, K. J., Steinert, J. J. and Trawatha, S. E. (1990), Seed priming influence on germination and emergence of pepper seed lots. *Crop Science* 30, pp. 718–721.

[7] Carrow, R. N. (1996), Drought resistance aspect of turf grasses in the South-East: root-shoot responses. *Crop Science* 36, pp. 687–694.

[8] De Villiers, A. J., Van Rooyrn, M. W., Theron, G. K., Van Deventer, H. A. (1994), Germination of three namaqualand pioneer species, as influenced by salinity, temperature and light. *Seed Science and Technology* 22, pp. 427–433.

[9] Demir, I. and Ellis, R. (1994), The effects of priming on germination and longevity of harvest pepper seed lots. *Turkish Journal of Agriculture and Forestry* 18, pp. 213–217.

[10] Fujikura, Y. and Karssen, C. M. (1992), Effects of controlled deterioration and osmopriming on protein synthesis of cauliflower seeds during early germination. *Seed Science Research* 2, pp. 23–31.

[11] Garcia, F. C., Jimenz, L. F. and Vazquez-Ramos, J. M. (1995), Biochemical and cytological studies on osmoprimed maize seeds. *Seed Science Research* 5, pp. 12–23.

[12] Ghassemi-Golezani, K., Khomari, S., Valizadeh, M. and Alyari, H. (2008), Effect of seed vigor and the duration of cold acclimation on freezing tolerance of winter oilseed rape. *Seed Science and Technology* 36, pp. 767–775.

[13] Harris, D., Joshi, A., Khan, P. A., Gothkar, P. and Sodhi, P. S. (1999), On-farm seed priming in semi-arid agriculture: Development and evaluation in maize, rice and chickpea in India using participatory methods. *Experimental Agriculture* 35, pp. 15–29.

[14] Hus, J. L. and Song, J. M. (1997), Antioxidant role of glutathione associated with accelerated aging and hydration of triploid watermelon seeds. *Physiologia Plantarum* 100, pp. 967–974.

[15] Jones, K. and Sanders, D. (1987), The influence of soaking pepper seed in water or potassium salt solutions on germination at three temperatures. *Journal of Seed Technology* 11, pp. 97–102.

[16] Kara, I. G. N. (1998), Response of wheat and barley during germination to seed osmopriming at different water potential. J. Agron. *Crop Science* 181, pp. 229–235.

[17] Kaur, S. A., Gupta, K., Kaur, N. (2006), Effect of hydro- and osmopriming of chickpea (*Cicerarietinum* L.) seeds on enzymes of sucrose and nitrogen metabolism in nodules. *Plant Growth Regulation* 49, pp. 177–182.

[18] Kaydan, D., Yagmur, M., Okut, N. (2007), Effect of salicylic acid on the growth and some physiological characters in salt stressed wheat (*Triticum aestivum* L.). *Tarim Bilimleri Dergisi* 13, pp. 114–119.

[19] Khan, A. A., Tao, K. L., Knyple, J. S., Brokowska, B. and Powell, L. E. (1978), Osmotic conditioning of seeds: Physiological and biochemical changes. *Acta Horticulturae* 83, pp. 267–278.

[20] Martin-Mex, R., Villanueva-Couoh, E., Herra-Campos, T. (2005), Positive effect of salicylates on the flowering of African Violet. *Scientia Horticulturae* 103, pp. 499–502.

[21] Moradi Dezfuli, P., Sharif-zadeh, P., Janmohammadi, M. (2008), Influence of priming techniques on seed germination behavior of maize inbred lines (Zea mays L.). *Journal of Agricultural and Biological Science* 3, pp. 23–25.

[22] Murungu, F. S., Nyamugafata, P., Chiduza, C., Clark, L. J., Whalley, W. R. (2003), Effects of seed priming aggregate size and soil matric potential on emergence of cotton (*Gossypium hirsutum* L.) and maize (*Zea mays* L.). *Soil and Tillage Research* 74, pp. 161–168.

[23] Omidi, H., Sorosh Zadeh, A., Salehi, A., Ghezli, F. D. (2005), Evaluation osmo-priming pre-soaking on rapeseed seed germination. *Agricultural Science Technology* 19, pp. 125–136.

[24] Rashid, P., Aollington, A., Harris, D., Khan, K. (2005), On-farm seed priming for barely on normal, saline and saline-sodic soils in North West Frontier Province, Pakistan. *European Journal of Agronomy* 24, pp. 276–281.

[25] Scarisbrick, D. H., Daniels, R. W., Noorrawi, A. B. (1982), The effect of chlormequat on the yield components of oil-seed rape (*Brassica napus* L.). *Journal of Agricultural and Biological Science* 99, pp. 453–455.

[26] Shad, K. K., John, G. M., Leigh, W. M. (2001), Germination of soybean seed primed in aerated solution of polyethylene glycol 8000. *Journal of Agricultural and Biological Science* 1, pp. 105–107.

[27] Shekari, F., Baljani, R., Saba, J., Afsahi, V. and Shekari, F. (2009), The effect of salicylic acid on growth characteristics of Borage (*Borago officinalis* L.). *New Agricultural Sciences* (in Persian) 6, pp. 47–53.

[28] Shekari, F., Javanshir, A., Shakiba, M. R., Moghaddam, M. and Alyari, H. (2000), Enhancement of canola seed germination and seedling emergence in low water potentials by priming. *Turkish Journal of Field Crops* 5, pp. 54–60.

[29] Subedi, K. D., and Ma, B. L. (2005), Seed priming does not improve corn yield in a Humid temperate environment. *Agronomy Journal* 97, pp. 211–218.

[30] Thakur, A., Thakur, P. S. and Bhardway, A. (1997), Influence of seed osmoconditioning on germination potential and seedling performance of bell pepper. *Seed Science Research* 25, pp. 25–30.

Acta Universitatis Sapientiae
Agriculture and Environment, **5**, (2013) 19–39

Ornamental plants as climatic indicators of arthropod vectors

Ákos BEDE-FAZEKAS[1]
email: bfakos@gmail.com

Attila János TRÁJER[2,3]
email: atrajer@gmail.com

[1]Corvinus University of Budapest, Faculty of Landscape Architecture,
Department of Garden and Open Space Design,
H-1118 Budapest, Villányi út 29-43

[2]University of Pannonia, Department of Limnology,
H-8200 Veszprém, Egyetem utca 10

[3]MTA-PE Limnoecology Research Group,
H-8200 Veszprém, Egyetem utca 10

Abstract. The importance and risk of vector-borne diseases (e.g., leishmaniasis, West Nile Virus, Lyme borreliosis) is going to increase in the European temperate areas due to climate change. Our previous studies have shown that the potential distribution of *Leishmania infantum* and some *Phlebotomus* (sand fly) species – a parasite of leishmaniasis, and its vectors – may be expanded even to the southern coastline of the Baltic Sea by the end of the 21st century. The lowland areas of the Carpathian Basin and the main part of Hungary are projected to be suitable for the studied sand fly vectors in the near future. It is important to find some indicator plants to examine whether the sand flies are able to live in a certain climate at a certain time. We studied several Mediterranean and Sub-Mediterranean plant species, and we found that the aggregated distribution of three ligneous species (*Juniperus oxycedrus* L., *Quercus ilex* L. and *Pinus brutia* Ten.) shows high correlation with the union distribution of five sand flies (*Phlebotomus ariasi* Tonn., *Ph. neglectus* Tonn., *Ph. perfiliewi* Parrot, *Ph. perniciosus* Newst. and *Ph. tobbi* Adler, Theodor et Lourie). Since these Mediterranean species are highly tolerant of the edaphic characteristics of the planting site, they may prove to be good indicators. The present and upcoming climate of Hungary is seen to be suitable for the selected indicator plant species, and it draws attention to and verifies the potential of the expansion of sand flies, which has been proved by some recent observations of the vectors in Southern Hungary.

Keywords: climate change, indicator plant species, climate envelope model, vector-borne diseases, leishmaniasis, sand fly

Introduction

Climate change and vector-borne diseases

The phenomenon of climate change has been accepted to a quite broad extent. There is a great amount of climate models based on the IPCC SRES climate scenarios, and free access is provided for them. The models are reliable enough and have good temporal and horizontal resolution for studying the potential future distribution of plant and animal vector species. Thus, the maps created by the model have importance not only for landscape architects and botanists (Czinkóczky and Bede-Fazekas 2012), but also for epidemiologists. By 2050, summer temperatures are predicted to increase by more than 2.5°C in the Mediterranean area, in Central Europe by less than 1.5°C and in Eastern Europe by about 1°C or less. The simulated warming is typically between 1.5°C and 2°C in most parts of Europe in winter. Although the precipitation in the Mediterranean area decreases by up to 50%, the precipitation increases in large, even in the northern parts of Europe in autumn and winter (Max-Planck-Institut 2007). As expected, the climate in the Carpathian Basin will be warmer, more arid, and will have extreme rainfalls more frequently in the colder half-year (Bartholy et al. 2007). Due to the climate change, the phenological, physiological and genetic parameters, the distribution area of the plant species and the stability of ecosystems seem to be going to change in the future (Hughes 2000, Kovács-Láng et al. 2008). Several predictions were made for the future distribution of plants, including the European species as well (Berry et al. 2006, Bakkenes et al. 2006, Harrison et al. 2006, Peterson et al. 2008, Bede-Fazekas 2012, Serra-Diaz et al. 2012).

An important impact of climate change on human health is the increasing hazard of vectorial diseases (animal-transmitted human diseases, mainly transported by arthropods: insects, ticks, etc.). The current importance of vectorial diseases is less in Europe than in the countries of the Third World, particularly in the Sub-Saharan Africa. The protective role of cold winters in the temperate climate may lose its importance. Due to the climate change, the importance of arthropod-borne diseases can increase by the end of the 21st century. Three groups of vectorial diseases can be discerned: 1) the recently abundant ones; 2) the diseases of the past centuries, which have disappeared but can appear again (re-emerging diseases); 3) the new, exotic diseases. The latter can be ranked among the Mediterranean or subtropical and tropical vectorial diseases. Lyme disease, tick-borne encephalitis and some other arthropod-borne diseases are good examples of the first group.

Vector-borne diseases are sensitive to climatic conditions (Rogers and Randolph 2006). Climate change can cause a shift in the geographical spread of insect populations (Ladányi and Horváth 2010) by modifying the climatic conditions and seasonal patterns and affecting the reproduction and the length of annual activity of vector species. These changes can increase their population in the recently inhabited areas, and by moderating the climate in the temperate areas of Europe, they can facilitate the migration of these arthropod vectors to the North (De la Roque et al. 2008). Changes in climatic patterns and in seasonal conditions may also affect disease behaviour in terms of spread pattern, diffusion range, amplification and persistence in novel habitats. Higher temperatures can induce the earlier flight of adult insects, eg., in the case of Lepidoptera species (Kocsis and Hufnagel 2011). Heat, humidity and sufficient organic matter are the main effectors of the larval development of sand flies (Naucke 2002, Lindgren and Naucke 2006), while the increasing CO_2 levels are usually unfavourably affecting the development of the insect larvae (Kocsis and Hufnagel 2011).

Leishmaniasis and sand flies

In the Subtropics and Tropics, leishmaniasis is one of the most important human vector-borne diseases with more than 12 million infected people (Naderer et al. 2006). *L. infantum* is the most notable causative agent of leishmaniasis in both humans and the reservoir animals in Europe (Ready 2010). Members of genus *Phlebotomus* (sand flies) are the main vectors of the unicellular eukaryote parasite genus Leishmania in Eurasia and Africa. Other sand fly vectors of *Leishmania* parasites can be found in the subgenera *Larroussius* and *Adlerius* (Killick-Kendrick 1990). In Southern Europe, leishmaniasis is mostly a zoonosis because the main hosts of *Leishmania* parasites are dogs and cats; however, foxes, rodents and horses can also be reservoirs (Sánchez et al. 2000; Pennisi 2002, Köhler et al. 2002, Solano-Gallego et al. 2003, Shaw et al. 2003) and a human-to-human transmission cycle is also possible (Alvar et al. 1997). The observed distribution of the cases of leishmaniasis in dogs (CanL) is similar to the human leishmaniasis' current occurrence (Lingren and

Naucke 2006, Solano-Gallego et al. 2011).

Phlebotomus species are characteristic Subtropical-Tropical faunal elements (Aspöck et al. 2008). The geographical distribution of leishmaniasis is limited by the distribution of the different sand fly species. *Phlebotomus ariasi*, *Ph. perniciosus*, *Ph. tobbi*, *Ph. perfiliewi* and *Ph. neglectus* are the main causative agents of *L. infantum*, while *Ph. papatasi* is the vector of *L. major*, *L. donovani*, *L. tropica*; *Ph. sergenti* can transmit *L. tropica* and *L. major*; *Ph. similis* is the main transmitter of *L. tropica* (Minter 1989, Killick-Kendrick 1990, WHO 1984, Léger et al. 2000). The vectors have a greater area than the parasite itself (Lindgren et al. 2008). In view of the above-mentioned fact, the modelled future expansion of sand flies does not imply the similar invasion of the parasites, too.

Indicator plant species

As it is recommended by the WHO (World Health Organization), using indicator species can help us study the effects of climate change. It has a high importance for the future to find some plant species that indicate the potential distribution of vectors and vectorial diseases. We aimed to bind some indicator plants to the Mediterranean-originated vectors. It is important to compare the reaction to climate change of the animal vectors of infectious diseases with the reaction of plant species. Since plants have a fixed position and they do not have the ability to produce notable heat like warm-blooded animals do (e.g., mammals), they are the most sensitive and therefore the most suitable climate indicators. Sand flies, which are able to move, can avoid climatic extremities in man-made (Killick-Kendrick and Killick-Kendrick 1987, Naucke 2002) and natural (Hanson 1961) shelters. In the case of plants, the climate affects them directly in their distribution area. Note that sand flies are also poikilothermic organisms as plants.

Ligneous plants were studied as indicator species instead of herbaceous plants since they are unable to react suddenly to the small-scale changes of climate. Thus, the natural distribution and the area of introduction of these species are strongly influenced by the extrema of climatic parameters. Therefore, their environmental demands can be modelled based on their current distribution as well. On the other hand, using plants as indicators instead of insects is much more ethical since their human-controlled introduction in the new sites is not hazardous.

Climate envelope model

We aimed to investigate the impact of climate change on the distribution of the selected vector and plant species with Climate Envelope Modelling (CEM) (Hijmans and Graham 2006). This method is about predicting species responses to climate change: it involves drawing an envelope around the domain of climate variables where a species is recently found and then identifying regions predicted to fall within that domain under scenarios for the future (Ibáñez et al. 2006). In contrast to mechanistic models, CEM (also known as niche-based modelling, correlative modelling) tries to find statistical correlations between the distribution of species and climate (Guisan and Zimmermann 2000, Elith and Leathwick 2009), and models the future temporal correspondence based on the present spatial correspondence between the variables (Pickett 1989). It postulates that (present and future) distribution is mostly dependent on the climate (Czúcz 2010), which is slightly questionable (Skov and Svenning 2004).

Various other ways can be found to determine the climate envelope, including simple regression, distance-based methods, genetic algorithms for rule-set prediction, and neural nets (Ibánez et al. 2006). About modelling vectorial diseases, see Peterson (2006).

Materials and methods

Climate data

The climate data were obtained from the regional climate model (RCM) REMO (ENSEMBLES 2013). The model REMO is based on the ECHAM5 global climate model (Roeckner et al. 2003, Roeckner et al. 2004) and the IPCC SRES A1B scenario. The A1B scenario supposes very fast economic increase, a worldwide population that peaks in the mid 21st century, and the introduction of innovative and efficient technologies (Nakicenovic and Swart 2000). The reference period of REMO is 1961–1990; the two future periods of modelling are 2011–2040 and 2041–2070. The horizontal resolution of the grid is 25 km. The entire European Continent is within the domain of REMO; we used however only a part (25724 of the 32300 points) of the grid. 36 climatic variables were averaged in the 30-year periods and used by the model: monthly mean temperatures (Tmean, °C), monthly minimum temperatures (Tmin, °C), and monthly precipitations (P, mm). Climate is the major driver of species distributions over Europe, and at the European coarse resolution land cover is mainly driven by climate, mainly by temperature and precipitation (Thuiller et al. 2004).

Distribution data

The studied species were *Leishmania infantum* Nicolle., *Phlebotomus ariasi* Tonn. (syn. *Larroussius* a.), *Ph. neglectus* Tonn. (syn. *Larroussius* n.), *Ph. perfiliewi* Parrot (syn. *Larroussius* p.), *Ph. perniciosus* Newst. (syn. *Larroussius* p.) and *Ph. tobbi* Adler, Theodor et Lourie (syn. *Larroussius t.*), and *Juniperus oxycedrus* L., *Pinus brutia* Ten. and *Quercus ilex* L. Our model was run on the distribution of parasite, vectors, and indicator plants separately. Since the model studied the climate requirements only of the European populations – the North African distribution segments were excluded –, it was able to project the shift of this part only.

The distribution data of *L. infantum* were derived from observations in 2003 (Trotz-Williams and Trees 2003). Only the continuous (not discrete) distribution data were taken into consideration where the force of infection was greater than zero. Weighting procedures were not used, the map of infection was reduced to a simple presence-absence map. The distribution in 2012 of the *Phlebotomus* species was obtained by VBORNET (2013). The regions entitled as 'indigenous' and 'recently present' were utilized with the same weight. All the *Leishmania* and *Phlebotomus* distribution data were based on the NUTS3 regions, which are the third-level public administration territories of the European Union. The distribution of *Juniperus oxycedrus*, *Pinus brutia* and *Quercus ilex* were derived from Tutin et al. (1964), EUFORGEN (2009) and Meusel et al. (1965), respectively. After a georeferencing process with 3^{rd} order polynomial transformation, the digitization of the bitmap-format distribution maps was realized with the assistance of the digital NUTS3 polygon borders (GISCO 2013).

Modelling method

Aggregated distribution of the eight studied Phlebotomus species was created, and the model for this union distribution was run iteratively to investigate the optimal amount of percentiles to be left from the climatic values. Cumulative distribution functions were calculated by PAST statistic analyser (Hammer et al. 2001) for the 36 climatic parameters. During the iterative modelling, 0+0 to 19+19 percentiles were left from the lower and higher values of a certain type of climate parameters (e.g., mean temperatures), while the other 2×12 climatic parameters were fixed at the extreme values (0-0 percentiles were left). In the meanwhile, two types of error values were calculated: 1) false negative, 2) false positive. They were summarized with the same weights divided between them. The point of minimum (the optimal number of percentiles to be left) of the accumulated error function was searched. It was found that the precipitation parameter drew quite different error functions than the temperatures, therefore, another iteration was run to study the difference between the lower and higher part of the precipitation percentiles. The two extrema of the minimum and mean temperatures were fixed, and iteratively more percentiles from the minimum of precipitation values were left while the maximum was fixed, and vice versa. The result of model calibration based on the aggregated *Phlebotomus* distribution was used during the modelling of the species.

Then the climatic data were refined by Inverse Distance Weighted interpolation method of ESRI ArcGIS 10 software. The modelling steps were as follows in the case of certain species: 1) the grid points within the distribution (a few thousand $\times$ 36 data) were queried; 2) the percentile points of the 36 climatic parameters (101$\times$36 data) were calculated; 3) the appropriate percentiles of the climatic parameters (2$\times$36 data) were selected; 4) modelling phrases (3 strings) were created by string functions of Microsoft Excel 2007 for the three modelling periods; 5) the areas were selected where all the climatic values of the certain period were between the extrema selected in step 3.

Results

Model calibration

The accumulated error functions showed that leaving percentiles from precipitation and from the minimum of precipitation results in graphs having the minimum point at the origin. Therefore, leaving percentiles from the minimum of precipitation values gives worse model than without percentile leaving. According to the result of iterations, 5-5 percentiles are to be left from the two extremes of mean temperature, 2-2 from the two extremes of minimum temperature, and 0 from the minimum and 8 from the maximum of precipitation.

Predicted distribution of the parasite

Observed and modelled distribution and predicted potential distribution of the parasite *L. infantum* is shown in Fig. 1. The recent distribution of visceral leishmaniasis is restricted to the Mediterranean coast line, mainly to the coastline of the Western Mediterranean Basin. The eastern Mediterranean area is highly vulnerable to *L. infantum*. Sporadic canine cases are known from Central Europe (Trotz-Williams and Trees 2003). The model predicted the potential distribution of the parasite with the sporadic cases in the reference period to be greater than the observed current distribution. The major difference can be observed in the eastern Mediterranean areas. Future expansion is expected principally in Asia Minor and the Balkan Peninsula, but the set of the affected countries is much larger: Spain, France and Hungary (mainly in the far future period), Serbia, Macedonia, Bulgaria, Romania, Ukraine and Turkey. Considering the current distribution and the model result, Southeast Europe and the Carpathian Basin are highly vulnerable areas. On the Italian coast, the Alps and the Pyrenees, the primary limiting value is the maximum of the precipitation in July.

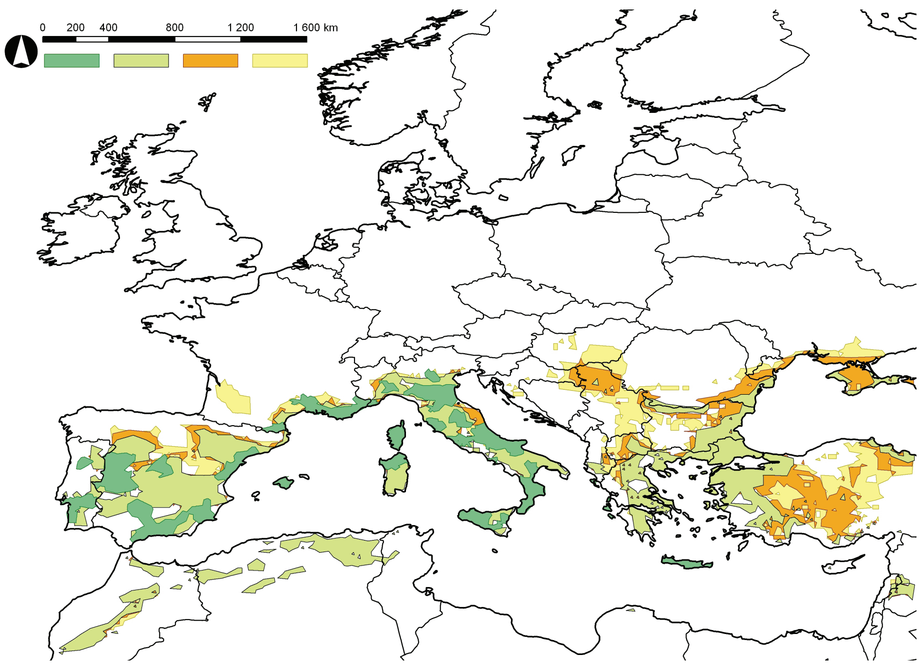

Figure 1: Current distribution (dark green), modelled potential distribution in the reference period (light green), and predicted potential distribution in the period of 2011–2040 (orange) and 2041–2070 (yellow) of the parasite *Leishmania infantum*. The figure is created by the authors with the assistance of ESRI ArcGIS.

Predicted distribution of the vectors

Observed and modelled distribution and predicted potential distribution of the aggregation of the five studied sand fly species are shown in Fig. 2. The Mediterranean, most of the territories of France and some regions with Sub-Mediterranean climate near the northern border are included in the observed distribution. The modelled potential distribution seems to be greater in Southeast and East-Central Europe. In the western regions, the observed and modelled distributions show more similarities. In the near future, period expansion is predicted mainly in Great Britain, Central Europe and Ukraine. In the period of 2041–2070, significant expansion is projected in Great Britain and in Ukraine.

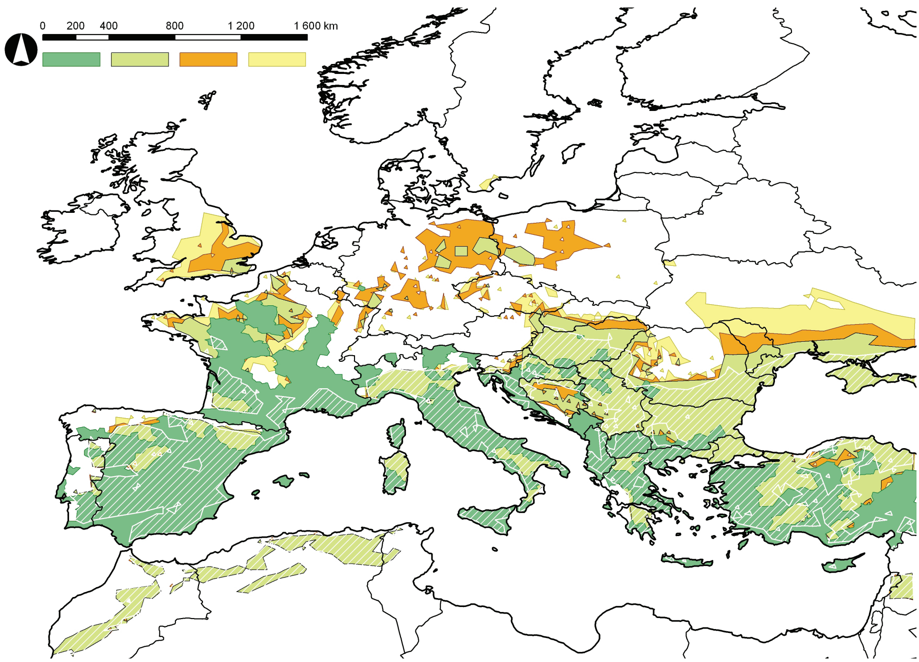

Figure 2: Current distribution (dark green), modelled potential distribution in the reference period (light green), and predicted potential distribution in the period of 2011–2040 (orange) and 2041–2070 (yellow) of the aggregated *Phlebotomus* species, and the modelled potential distribution of the aggregated indicator species in the reference period (white hatch). The figure is created by the authors with the assistance of ESRI ArcGIS.

Predicted distribution of the indicator plants

The aggregated observed and modelled (Fig. 2.) distribution – and the climatic requirements – of *Juniperus oxycedrus*, *Quercus ilex* and *Pinus brutia* show significant resemblance with those of the studied *Phlebotomus* species. Hence it can be stated that these three plant species can serve as climatic indicators of the vectors of *L. infantum*. The observed distribution gives us an inkling that these indicators can tolerate the winter mean temperature not lower than 0°C, their cold tolerance is, however, known to be greater. According to the hardiness zones of USDA, *Juniperus oxycedrus* and *Quercus ilex* are hardy to -17.7°C and *Pinus brutia* is hardy to -12.2 °C. The modelled potential distribution in the reference period is larger than the observed one mainly in Eastern Europe. Territories with -1 – -2°C mean temperature in January are included. The predicted future distributions are not much more expanded than in the reference period. Expansion seems to be occurred in Northwestern France, South England and the Carpathian Basin.

Discussion

Opinions differ if climate is by itself sufficient or even the most important factor for explaining species distributions (Dormann 2007). According to Kennewick et al. (2010), the most important limit of the distribution of sand flies is the winter average and minimum temperatures and the cold and rainy summers. Note that absolute climatic values and extremes rather than averages may explain the limits of distribution better (Kovács-Láng et al. 2008). In contrary to the animals, the distribution of plants is able to be limited by not only the climatic but the edaphic parameters, too. Thus, it was important to examine whether the selected indicator species have wide tolerance to the soil type. In the distribution area of the studied species, numerous different soil types can be observed, including leptosols, regosols, luvisols, cambisols, calcisols, fluvisols, vertisols and umbrisols (FAO 1971). While almost all the soil types of Central and Western Europe are found within the current distribution area, the edaphic parameters will probably not limit the future expansion of these species.

Future predictions for the periods of 2011–40 and 2041–70 show similarities in the case of the indicators and vectors. The latter is somewhat greater, primarily in Central Europe, Germany and Poland. It should be noted that some distinct territories in Germany, South England and Poland are included in the modelled potential distribution for the reference period, which is in accordance with the observation of Fischer et al. (2010). In the Carpathian Basin and Eastern Europe, the modelled distribution shows significant similarities to that of the vectors (Fig. 3.).

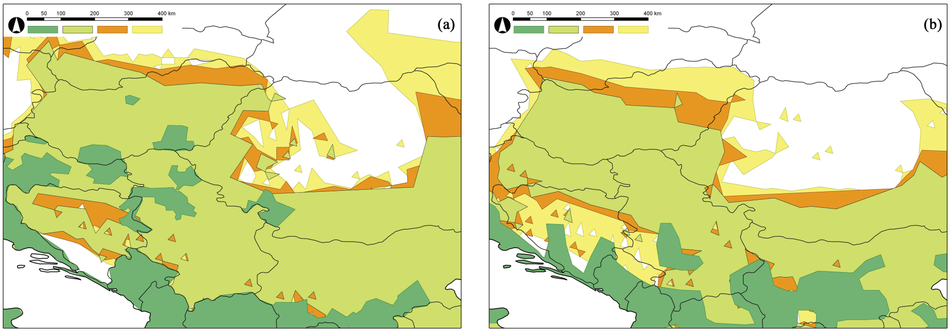

Figure 3: Comparison of the model results of sand flies (a) and indicator plant species (b) zoomed to the Carpathian Basin. The figure is created by the authors with the assistance of ESRI ArcGIS and Adobe Photoshop.

In the Carpathian Basin, the northern border of the modelled distributions coincides with the northwestern-southeastern winter isotherms. The distance of the northern border of the indicator and vector species is 50–150 km; this difference is increasing towards the east. In Eastern Europe, the distribution borders and the isotherms have a west-east direction. The above-mentioned distance is larger to the east of the Carpathians than in the Carpathian Basin. In Transylvania, the presence of the indicators is not predicted for the studied periods. Climate in Northern Bosnia and Herzegovina is predicted to become suitable for the vectors by 2040, but for the indicators only by 2070.

The selection of the indicators was verified by Cohen's kappa measurement based on the distribution of the vectors and the indicators. Since the Cohen's kappa value in the case of the modelled distributions (0.7938) is much greater than in the case of the observed distributions (0.6057), the similarities in the climatic requirements are greater than it was expected based on the observed distributions.

It was concluded that the studied plant species can be reckoned as indicators of sand flies. Hence, in the regions where young specimens of these species are able to survive without any (e.g., frost) protection, the appearance of the vectors is expected. Therefore, Hungary is highly endangered (Fig. 4., Fig. 5., Fig. 6.), which is in accordance with the recent observation of sand flies in Southern Hungary (Farkas et al. 2011).

Figure 4: *Pinus brutia* specimen in Budapest. The photo was taken by the authors in 2010.

Figure 5: *Quercus ilex* specimen in Pécs. The photo was taken by the authors in 2011.

Conclusions

1. It was discussed that vector-borne diseases originated in the Mediterranean can endanger the temperate part of Europe.
2. A model was calibrated with iterative error evaluation to study the future expansion of *Leishmania infantum*, its five sand fly vectors and three plant species.
3. The model was run for the reference period (1961–90) and for the two prediction periods (2011–40, 2041–70), and maps were drawn based on the model results.
4. The aggregated distribution of *Juniperus oxycedrus*, *Pinus brutia* and *Quercus ilex* was proven to be able to serve as a climate indicator of the aggregation of sand flies.
5. It was concluded that Hungary is highly endangered by the *Phlebotomus* species, and therefore by *Leishmania infantum*.

Figure 6: Young *Juniperus oxycedrus* specimen in Budakeszi. The photo was taken by the authors in 2009.

Acknowledgements

Special thanks to Anna Páldy (National Institute of Environmental Health), János Bobvos (National Institute of Environmental Health), Levente Horváth (Corvinus University of Budapest) and Levente Hufnagel (Corvinus University of Budapest) for their selfless assistance. The research was supported by Project TÁMOP-4.2.1/B-09/1/KMR-2010-0005 and TÁMOP-4.2.2.A-11/1/KONV-2012-0064. The ENSEMBLES data used in this work was funded by the EU FP6 Integrated Project ENSEMBLES (Contract number 505539), whose support is gratefully acknowledged.

References

[1] Alvar J., Canavate, C., Gutiérrez-Solar, B., Jiménez, M., Laguna, F., López-Vélez, R., Molina, R., Moreno, J. (1997), Leishmania and human immunodeficiency virus coinfection: the first 10 years. Clin. Microbiol. Rev., 10(2): 298–319.

[2] Aspöck, H., Gerersdorfer, T., Formayer, H., Walochnik, J. (2008), Sandflies and sandfly-borne infections of humans in Central Europe in the light of climate change. Wiener klinische Wochenschrift, 120(4): 24–29.

[3] Bakkenes, M., Eickhout, B., Alkemade, R. (2006), Impacts of different climate stabilisation scenarios on plant species in Europe. Global Environmental Change, 16(1): 19–28.

[4] Bartholy, J., Pongrácz, R., Gelybó, Gy. (2007), A 21. század végén várható éghajlatváltozás Magyarországon. Földrajzi Értesítő, 56(3-4): 147–168.

[5] Bede-Fazekas, Á. (2012), Melegigényes díszfák telepíthetőségi területének előrejelzése a 21. századra. Thesis, Corvinus University of Budapest, Faculty of Landscape Architecture, Budapest.

[6] Berry, P. M., Rounsevell, M. D. A., Harrison, P. A., Audsley, E. (2006), Assessing the vulnerability of agricultural land use and species to climate change and the role of policy in facilitating adaptation. Environmental Science & Policy, 9(2): 189–204.

[7] Czinkóczky, A., Bede-Fazekas, Á. (2012), Visualization of the climate change with the shift of the so-called Moesz-line. In: Buhmann, E.,

Ervin, S., Pietsch, M. (eds.): Peer Reviewed Proceedings of Digital Landscape Architecture 2012 at Anhalt University of Applied Sciences. Herbert Wichmann Verlag, Berlin, pp. 437–444.

[8] Czúcz, B. (2010), Az éghajlatváltozás hazai természetközeli élőhelyekre gyakorolt hatásainak modellezése. PhD dissertation. Corvinus University of Budapest, Faculty of Horticultural Sciences. Budapest.

[9] De la Roque, S., Rioux, J. A., Slingenbergh, J. (2008), Climate change: Effects on animal disease systems and implications for surveillance and control. Revue Scientifique Et Technique. International Des Epizooties, 27(2): 339–354.

[10] Dormann, C. F. (2007), Promising the future? Global change projections of species distributions. Basic and Applied Ecology, 8(5): 387–397.

[11] Elith, J., Leathwick, J. R. (2009), Species Distribution Models: Ecological Explanation and Prediction Across Space and Time. Annual Review of Ecology, Evolution, and Systematics, 40(1): 677–697.

[12] ENSEMBLES (2013), ENSEMBLES data archive. ensemblesrt3.dmi.dk. Last accessed: 2013.03.01.

[13] EUFORGEN (2009), Distribution map of Brutia pine (Pinus butia). www.euforgen.org/distribution_maps.html. Last accessed: 2013.01.01.

[14] FAO (1971): FAO-UNESCO Soil Map of the World, 1:500 000. Food and Agriculture Organization, United Nations, Rome and Paris.

[15] Farkas, R., Tánczos, B., Bongiorno, G., Maroli, M., Dereure, J., Ready, P. D. (2011), First surveys to investigate the presence of canine leishmaniasis and its phlebotomine vectors in Hungary. Vector Borne Zoonotic Dis, 11(7): 823–834.

[16] Fischer, D., Thomas, S. M., Beierkuhnlein, C. (2010), Temperature-derived potential for the establishment of phlebotomine sandflies and visceral leishmaniasis in Germany. Geospatial Health, 5(1): 59–69.

[17] GISCO (2013), GISCO - Eurostat (European Commission). epp.eurostat.ec.europa.eu/portal/page/portal/gisco_Geographical_information_maps/popups/references/ administrative_units_statistical_units_1. Last accessed: 2013.01.01

[18] Guisan, A., Zimmermann, N. E. (2000), Predictive habitat distribution models in ecology. Ecological Modelling, 135(2-3): 147–186.

[19] Hammer, Ř., Harper, D. A. T., Ryan, P. D. (2001), PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica, 4: 9.

[20] Hanson, W. J. (1961) The Breeding Places of Phlebotomus in Panama (Diptera, Psychodidae). Annals of the Entomological Society of America, 54(3): 317–322.

[21] Harrison, P. A., Berry, P. M., Butt, N., New, M. (2006), Modelling climate change impacts on species' distributions at the European scale: implications for conservation policy. Environmental Science & Policy, 9(2): 116–128.

[22] Hijmans, R. J., Graham, C. H. (2006), The ability of climate envelope models to predict the effect of climate change on species distributions. Global Change Biology, 12(12): 2272–2281.

[23] Hughes, L. (2000), Biological consequences of global warming: is the signal already apparent? Trends in Ecology and Evolution, 15(2): 56–61.

[24] Ibánez, I., Clark, J. S., Dietze, M. C., Feeley, K., Hersh, M., Ladeau, S., Mcbride, A., Welch, N. E., Wolosin, M. S. (2006), Predicting Biodiversity Change: Outside the Climate Envelope, beyond the Species-Area Curve. Ecology, 87(8): 1896–1906.

[25] Kennewick, W. A., Marfin, A. A. (2010), Emerging Vector-Borne Infectious Diseases What's New in Medicine Workshop.

[26] Killick-Kendrick, R. (1990), Phlebotomine vectors of the leishmaniases: a review. Medical and Veterinary Entomolog, 4(1): 1–24.

[27] Killick-Kendrick, R., Killick-Kendrick, M. (1987), The laboratory colonization of Phlebotomus ariasi (Diptera, Psychodidae). Ann Parasitol Hum Comp, 62(4): 354–356.

[28] Kocsis, M., Hufnagel, L. (2011), Impacts of climate change on Lepidoptera species and communities. Applied Ecology and Environmental Research, 9(1): 43–72.

[29] Köhler, K., Stechele, M., Hetzel, U., Domingo, M. Schönian, G., Zahner, H., Burkhardt, E. (2002), Cutaneous leishmaniosis in a horse in southern Germany caused by Leishmania infantum. Vet Parasito, 16(109): 9–17.

[30] Kovács-Láng, E., Kröel-Dulay, Gy., Czúcz, B. (2008), Az éghajlatváltozás hatásai a természetes élővilágra és teendőink a megőrzés és kutatás területén. Természetvédelmi Közlemények, 14(1): 5–39.

[31] Ladányi M., Horváth, L. (2010), A review of the potential climate change impact on insect populations - general and agricultural aspects. Applied Ecology and Environmental Research, 8(2): 143–152.

[32] Léger, N., Depaquit, J., Ferté, H., Rioux, J. A., Gantier, J. C., Gramiccia, M., Ludovisi, A., Michaelides, A., Christophi, N., Economides, P. (2000), Phlebotomine sandflies (Diptera: Psychodidae) of the isle of Cyprus. II - isolation and typing of Leishmania (Leishmania infantum Nicolle, 1908 (zymodeme MOM 1) from Phlebotomus (Larrouius) tobbi Adler and Theodor, 1930. Parasite, 7(2): 143–146.

[33] Lindgren, E., Naucke, T. (2006), Leishmaniasis: Influences of Climate and Climate Change Epidemiology, Ecology and Adaptation Measures. In: Menne, B, Ebi, K. L. (eds.): Climate change and adaptation strategies for human health. Steinkopff Verlag, Darmstadt, pp. 131–156.

[34] Lindgren, E., Naucke, T., Menne, B. (2008), Climate Variability And Visceral Leishmaniasis In Europe. WHO/TDR Working paper for the Scientific Working Group meeting on Leishmaniasis Research, convened by the Special Programme for Research and Training in Tropical Diseases, Geneva.

[35] Max-Planck-Institut für Meteorologie (2007), What will the climate in Europe look like in the middle of the 21st century? www.mpimet.mpg.de/en/news/press/faq-frequently-asked-questions/what-will-the-climate-in-europe-look-like-in-the-middle-of-the-21st-century.html. Last accessed: 2013.03.01.

[36] Meusel, H., Jäger, E. J., Weinert, E. (1965), Vergleichende Chorologie der zentraleuropäischen Flora. Band I. (Text und Karten). Fischer-Verlag, Jena.

[37] Minter, D. M. (1989), The leishmaniasis. In: Geographical distribution of arthropod-borne diseases and their principal vectors. WHO, Geneva (document WHO/VBC/89.967)

[38] Naderer, T., Ellis, M. A., Sernee, M. F., De Souza, D. P., Curtis, J., Handman, E., McConville, M. J. (2006), Virulence of Leishmania major in macrophages and mice requires the gluconeogenic enzyme fructose-1,6-bisphosphatase. PNAS. 103(14): 5502–5507.

[39] Nakicenovic, N., Swart, R. (eds.) (2000), Emissions Scenarios. Cambridge University Press, Cambridge.

[40] Naucke, T. J. (2002), Leishmaniosis, a tropical disease and its vectors (Diptera Psychodidae, Phlebotominae) in Central Europe. Denisia. 6: 163–178.

[41] Pennisi, M. G. (2002), A high prevalence of feline leishmaniasis in southern Italy. In: Killick-Kendrick, R. (ed.): Canine leishmaniasis: moving towards a solution. Proceedings of the Second International Canine Leishmaniasis Forum Seville, Spain. Intervet International, Boxmeer, The Netherlands. pp. 9–48.

[42] Peterson, A. T. (2006), Ecological niche modeling and spatial patterns of diseases transmission. Emerging Infectious Diseases, 12(12): 1822–1826.

[43] Peterson, A. T., Stewart, A., Mohamed, K. I., Araújo, M. B. (2008), Shifting Global Invasive Potential of European Plants with Climate Change. PLoS ONE, 3(5): e2441.

[44] Pickett, S. T. A. (1989): Space-for-time substitution as an alternative to long-term studies. In: Likens, G. E. (ed.): Long-Term Studies in Ecology: Approaches and Alternatives. Springer, New York. pp. 110–135.

[45] Ready, P. D. (2010), Leishmaniasis emergence in Europe. Euro Surveill, 15(10): 19505.

[46] Roeckner, E., Bäuml, G., Bonaventura, L., Brokopf, R., Esch, M., Giorgetta, M., Hagemann, S., Kirchner, I., Kornblueh, L., Manzini, E., Rhodin, A., Schlese, U., Schulzweida, U., Tompkins, A. (2003), The atmospheric general circulation model ECHAM 5. Part I: Model description. Max-Planck-Institut für Meteorologie, Hamburg.

[47] Roeckner E., Brokopf, R., Esch, M., Giorgetta, M., Hagemann, S., Kornblueh, L., Manzini, E., Schlese, U., Schulzweida, U. (2004), The atmospheric general circulation model ECHAM 5. PART II: Sensitivity of Simulated Climate to Horizontal and Vertical Resolution. Max-Planck-Institut für Meteorologie, Hamburg.

[48] Rogers, D. J., Randolph, S. E. (2006), Climate Change and Vector-Borne Diseases. Advances in Parasitology, 62: 345–381.

[49] Sánchez, M., Hervás, J., Chacón, F., Gómez, J., Luicentes, J., Castrillo, J., Pérez, R., Pascual, F., Pascual, F. (2000), Evaluación del gato común (Felis catus domesticus) como reservorio dela leishmaniosis enela cuenca mediterranea. Revista Técnica Veterinaria, Pequenos Animales. 24: 46–54

[50] Serra-Diaz, J. M., Ninyerola, M., Lloret, F. (2012), Coexistence of Abies alba (Mill.) - Fagus sylvatica (L.) and climate change impact in the Iberian Peninsula: A climatic-niche perspective approach. Flora - Morphology, Distribution, Functional Ecology of Plants, 207(1): 10–18.

[51] Shaw, S. E., Lerga, A., Williams, S. (2003), Review of exotic infectious diseases in small animals entering the United Kingdom from aboard diagnosed by PCR. Vet. Rec., 152(6): 176–177.

[52] Skov, F., Svenning, J. C. (2004), Potential impact of climatic change on the distribution of forest herbs in Europe. Ecography, 27(3): 366–380.

[53] Solano-Gallego, L., Fernández-Bellon, H., Serra, R., Gállego, M., Ramis, A., Fondevila, D., Ferrer, L. (2003), Cutaneous leishmaniosis in three horses in Spain. Equine Vet J., 35(3): 320–323.

[54] Solano-Gallego, L., Guadalupe, M., Koutinas, M., Cardoso, L., Pennisi, M. G., Ferrer, L., Bourdeau, P., Gaetano, O., Baneth, G. (2011), LeishVet guidelines for the practical management of canine leishmaniosis. Parasites & Vectors, 4: 86.

[55] Thuiller, W., Araújo, M. B., Lavorel, S. (2004), Do we need land-cover data to model species distributions in Europe? Journal of Biogeography, 31(3): 353–361.

[56] Trotz-Williams, L. A., Trees, A. J. (2003): Systematic review of the distribution of the major vector-borne parasitic infections in dogs and cats in Europe. Veterinary Record, 152: 97–105.

[57] Tutin, T. G., Burges, N. A., Chater, A. O., Edmondson, J. R., Heywood, V. H., Moore, D. M., Valentine, D. H., Walters, S. M., Webb, D. A., Akeroyd, J. R., Newton, M. E., Mill, R. R. (1964), Flora Europaea. - Cambridge University Press, Cambridge. VBORNET (2013),

[58] VBORNET maps - Sandflies. ecdc.europa.eu/en/activities/disease-programmes/emerging_and_vector_borne_diseases/pages/ vbornet_maps _sandflies.aspx?MasterPage=1. Last accessed: 2013.01.01.

[59] WHO (1984): The leishmaniases: report of an expert committee. WHO Tech Rep Ser 701: 1–140.

Acta Universitatis Sapientiae
Agriculture and Environment, **5**, (2013) 40–55

The settling factors of Roman villas in southern Lusitania

Anett FIRNIGL
email: anett.firnigl@gmail.com

Corvinus University of Budapest,
Faculty of Landscape Architecture,
Department of Garden Art,
29-43. Villányi Str., H-1118 Budapest

Abstract. The Romans arrived to the Iberian Peninsula in the 3rd century B.C.: they transformed the Hispanian administration, the landscape and culture. The area of Lusitania expanded in the middle and southern part of Portugal, south from the River Douro, as well as on the autonom community of Extremadura, Spain. The production of the Roman villas gave the great mass the agricultural and commercial background of the Province. These produced wares got to the several lands of the Empire on the well-established road network and across the rivers and seas. The Roman *villa* was on a cultivation- and stock-raising-adapted farming unit with living houses, bath, and outbuildings, which had the biggest importance. The *villas* of Lusitania were concetrated into several groups: around the cities of Cascais and Lisboa, Évora and Mérida, as well. A bigger group ran along the southern coast of the Iberian Peninsula: the *villas* presented in this study (e.g., Milreu, Cerro da Vila, and Abicada) were specialized on seafood products and maritime trade. Other sites are also known where the presence of a villa has not been discovered yet, but where economic and industrial facilities were excavated (e.g., *cetária*, which means a basin for the production of the fish sauce *garum* in the Portuguese terminology).

Keywords: Iberian Peninsula, landscape, trade, pars urbana and rustica, villa maritima

Introduction. The appearance of the Romans on the Iberian Peninsula

The first Roman army corps arrived at the Iberian Peninsula in 219 B.C. to wage war against the Carthaginians, who were recruiting Hispanian allies for the Second Punic War. The war ended with Roman victory: they extended their power almost on the whole peninsula, systematically occupying the area from south to north (Teichner 2005, Saraiva 2010). The Romans transformed the Hispanian administration, too: they organized the peninsula under two, later three provinces: Tarraconensis occupied the biggest area of the peninsula, Baetice was located on the southern edge and Lusitania along the *Oceanus Atlanticus* (Atlantic Ocean). Gallaecia was later separated from Tarraconensis at the north-western part of the peninsula and then Carthaginensis at the southern part at the end of the 3rd century (see Figure 1).

Figure 1: The administrative subdivision of Hispania in the Roman Age: in the Republic (a), in the Principate (b) and after Diocletian's reorganization in the 3rd century (c)

The area of Lusitania expanded in the middle and southern part of Portugal, south from the River Douro, as well as on the autonom community of Extremadura, Spain. In this study, those villas on the southern shore of Lusitania are presented which were specialized mainly on maritime trade.

Materials and methods. The natural conditions and settlements of Lusitania

The high level of industrial and agricultural production, the trade and financial systems, the advanced urban culture and the fine arts mean the culture of the Roman Empire. In addition, a key factor was the well-developed road network, the regular army, the written law and the Latin language. In the beginning, Lusitania had an importance for the Empire from a military point of view and it had a significant role in agricultural and handicraft production as well as in trading with Italy and other provinces (Saraiva 2010, Firnigl 2012, Moutinho Alarcão 1997). The Roman activity transformed the Hispanian landscape: they opened quarries and built military camps, cities with paved streets and villas from the excavated materials. These settlements were connected together with roads, which were sorrunded by cemeteries. The Romans exterminated forests, drained the moorlands and created ponds by building dams (Saraiva 2010, Firnigl 2012, Moutinho Alarcão 1997).

The natural and climatic conditions of Lusitania

The Tagus (today River Tejo) divided the area of the former Lusitania into two major areas and it was bordered by the Durius (today River Douro) on the north. The third major river of Lusitania was the Monda (today River Mondego), which flows through the city of Coimbra. The area bounded by the rivers Tagus and Durius is mountainous, including some parts of the Sierra de Estréla, the highest mountain in Central Portugal, reaching almost 2000 meters. The area south from the Tagus has plains and plateaus, and its coast has alternate rocky and sandy parts. The two regions of Southern Portugal are the Alentejo, with an approximated 400-m altitude, and Algarve at the southern coast, presented here in more details.

The raw materials of the quarries and mines with the workshops were one of the most important economic factors of Roman Portugal, especially in the northern areas (Edmondson 1989, Moutinho Alarcão 1997). The number of mines in the southern parts is lower; there are only seven sites in the municipalities examined here (there are four mines in the municipality of Silves, mainly copper mines, two in Loulé, and only one in Albufeira): these mines are in the ca. 200–250-m high regions (Marques 1992; see Figure 2).

Today, the climate of the coast in Southern Lusitania is Mediterranean, where the annual mean temperature is 18°C. South from the Tejo, the annual rainfall is 500–700 mm and in Algarve it is only ca. 450 mm. The continental type of the climate is stronger from the coasts towards the middle of the country; rainfall highly increases in the northernmost parts. The Roman dams were built where the average annual rainfall was relatively low: most of the dams were in the areas under ca. 800 mm rain and under ca. 600 mm at Southern Portugal (Butzer et al. 1985, Marques 1992, Moutinho Alarcão 1997). The water storage capacity of streams swollen by the dams reached several hundred thousand cubic meters: the dam of Lameira (Vila Velha da Ródão) kept 840,000 m^3 of water (Moutinho Alarcão 1997).

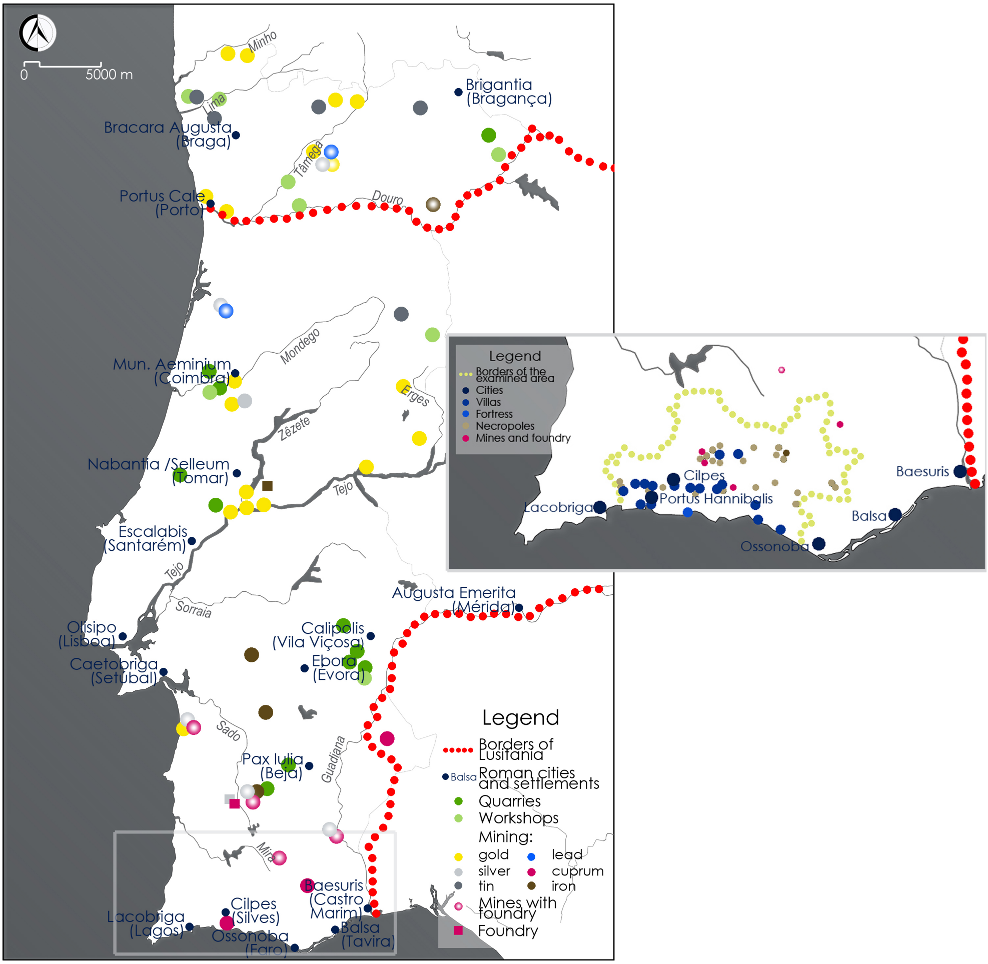

Figure 2: The mines and quarries of Roman Portugal, with the details of the examined area (after Marques 1992 and Moutinho Alarcão 1997, p. 80. and p. 96.)

The dams were also connected to the villas: the Romans covered the water from the swollen waters for the irrigation of gardens and fields, for watering animals and for the water supply of baths as well (Firnigl 2012, Moutinho Alarcão 1997; see Figure 3).

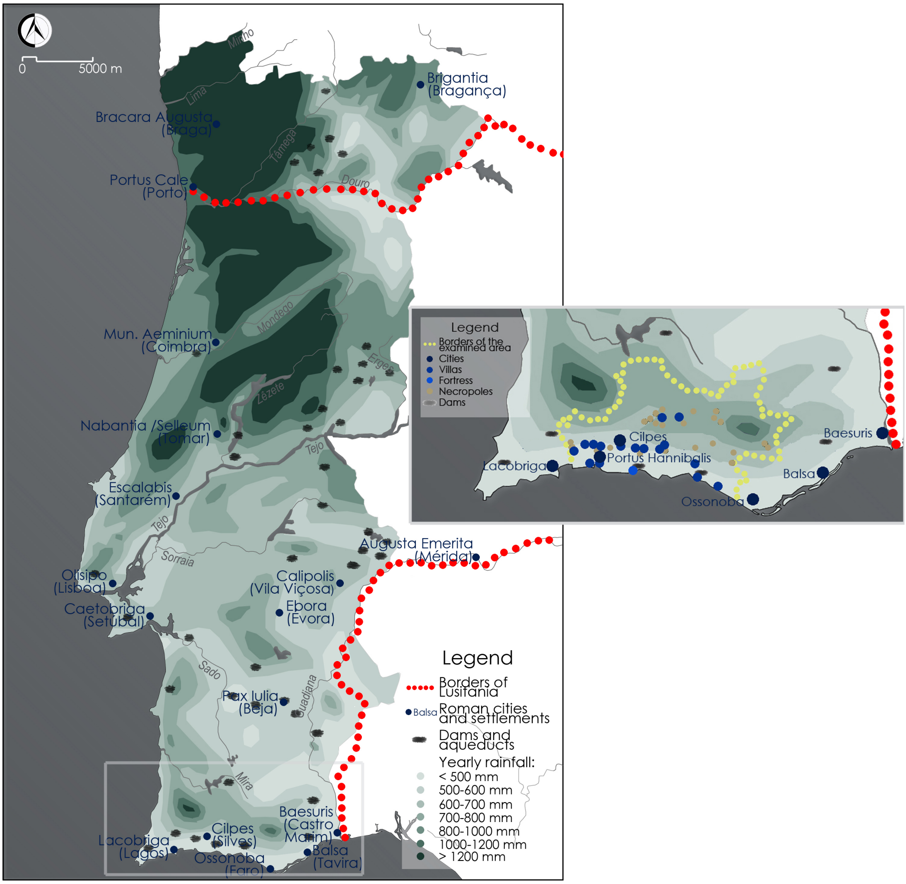

Figure 3: The dams and aqueducts of Roman Portugal with the details of the examined area (after Marques 1992 and Moutinho Alarcão 1997, p. 18.)

The settlement network of Lusitania

According to Pliny's description in the 1st century, Lusitania was fragmented into three so-called conventus (Roman judicial and administrative units): their centres were Augusta Emerita (today Mérida, Spain), Pax Iulia (today Beja, Portugal) and Escalabis (today Santarém, Portugal). A total of 45 urban settlements were created here (Saraiva 2010), which were linked with a well-developed road network (Thompson 1918). The directions of the main roads are still identifiable: these roads facilitated the freight transport and several bridges were maintained over the streams (Marques 1992, Saraiva 2010; see Figure 4).

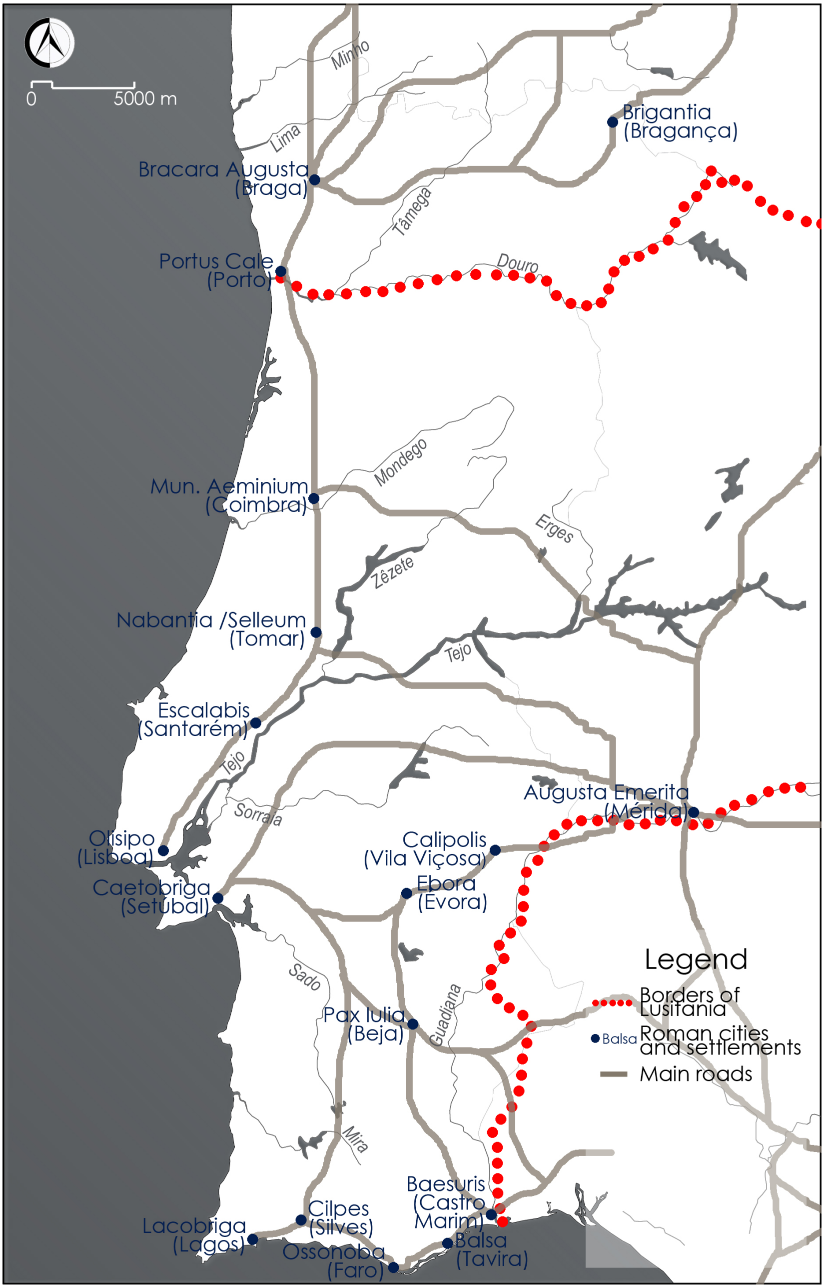

Figure 4: The main roads of Roman Portugal

Olisipo (today Lisboa, the capital of Portugal) grew into a large port city at the beginning of the period of the Roman Empire: it was a node from where the local products were transported to Italy. The culitvation of cereals contributed to the development of the towns of Roman origins, Santarém, Évora, Beja and Alcácer do Sal as well (Saraiva 2010). The most important towns and settlements on the southern shore were Balsa (today Tavira), the port city of Ossonoba (today Faro) and Cilpes (today Silves), Lacobriga (Lagos), Baesuris (Castro Marim), Portus Hannibalis (vicinity of Portimão), Ipses (Alvor) and also Conistorgis (unknown place). Many *villas* were established in the districts of these towns: 18 villas and seven rural settlements can be identified on the southern coast, examined here. Besides them, three other sites can be determined as villas, where only economic or agricultural buildings (e.g., silo, press house) were found. A fortress is also known on the southern coast, as well as 27 cemeteries or graves, where the connected settlements are not known yet (Marques 1992, Teichner 2005).

The meaning of 'villa' and the Lusitanian villas

The word *villa* is a generic term without architectural meaning originally (see B. Thomas 1961, Mócsy and Fitz 1990, Marzano 2007, Moutinho Alarcão 1997): it does not mean holiday house in the modern sense, but a farming unit producing for the local market, with residential houses, outbuildings (e.g., barns, stables, sheds) and gardens (vegetable garden, orchards and flower gardens). These units were distinguished (see B. Thomas 1961, Mócsy and Fitz 1990) with the names *pars urbana* (the residential part with the owner's or tenant's main building and baths) and *pars rustica* (with two or more outbuildings). The *pars rustica* had significance originally: Cato explained all of its elements, but he noted about the *villa urbana* that it had to be constructed according to the owner's financial opportunities (*villam urbanam pro copia aedificat*; see Mócsy and Fitz 1990). The focus was on the production in Italy and especially in the provinces, which took place in the villas.

The centres of the farms were usually enclosed with wooden or stone walls (Mócsy 1995), but they were not primarily built for defensive purposes: these walls were built as fences to indicate the boundaries of the manor, separating the fields and pastures from the *pars urbana* and *rustica*, to hold the crops, tools and animals in the central yard (K. Palágyi 1994, Mócsy 1995). Fields belonged to the *villas* in the original meaning, even so closely that a parcel could have the name *fundus* only in the case when a *villa* stood on it (Mócsy and Fitz 1990). The quantity and quality of buildings, the size of the main building and the fenced central yard probably refer to the size of the property: the *auctors*' requirement was that the *villa* had to be proportional with the size of the *fundus*. According to this, small (2.5–50 acres area), medium-sized (20–125 acres) and large (over 125 acres) estates can be distinguished (see Hainz Dohr's estate categories, White 1966). The size of the estates could depend on the location inside the province, on natural and terrain conditions, and on the production methods as well.

The examination of Hispanian *villas* is thourough, especially in the Spanish areas, but the researches in Portugal are in disatvantage: Jean-Gérard Gorges's inventory is the most detailed work from 1979 on; however, he rather specialized in Spanish sites. The archaeological-topographical series is the most recent summary, edited by Teresa Marques. The *villas* of Lusitania were concetrated in several groups: the first major group is observed around the cities of Cascais and Lisboa; furthermore, several villas surround Évora and Mérida as well. A bigger group runs through the southern coast of the Iberian Peninsula: these *villas* were specialized in seafood products and maritime trade. The typical territory of the estates is around 200 acres (large estates) at the Alentejo, but only 20–50 acres (medium-sized estates) in the Northern Lusitania (Saraiva 2010. Moutinho Alarcão 1997). This north-south difference still remained at the beginning of the 20$^{\text{th}}$ century, too (Thompson 1918).

The meaning of the *villa* changed later, to the late Imperium (B. Thomas 1961, Mócsy and Fitz 1990); various terms were separated by the function and location of *villas*. Thus, the so-called *refugium villas* were built for defense against the barbarian attacks. The *villa publica* was a wayside buy-in restaurant, a station for log change (*mutatio*). The *villa suburbana* was independent from the fields; it is the collective name of peri-urban luxury homes built for vacation (this word is the ancestor of the modern villa concept). The *villa pseudourbana* had an estate, but the closed, inward-looking main building was built after the model of urban residences (B. Thomas 1961, Castiglione 1971, 1973, McKay 1980, Vitruvius 1988). The villa maritima has to be mentioned here, which, contrary to the *villa rustica*, was a rich building with an opened front to the seas, appearing also in Lusitania (Marzano 2007).

The villas were typically Roman units, so they are not known from pre-Roman times (B. Thomas 1961). Only the Romans and veterans were living in this architectural unit initially, then also the natives got more and more fields with the romanization (B. Thomas 1961). The constructions of the ancient Romans followed simplicity plans, the settlements and buildings were established in the provinces by basic types modelled in Italy (Mócsy 1974, Mócsy and Fitz 1990). These schemes can be discovered at the *domus*, too;

the Roman houses were built on the same type, which were modified mostly according to the local climate. These types of houses were taken over for construction of villas as well. Two main types can be distinguished with the layout of main residential buildings, although these types appeared clearly rarely. The rooms of *porticus villas* or *villas* with central corridors were organized in linear order, and they had usually smaller areas. The villas with *peristylium* or with central patio had bigger areas (B. Thomas 1961, Mócsy and Fitz 1990, Reutti 1994, Bechert 2005): in the following examined villas, mostly this type of patio can be found.

The German terminology uses two words to differenciate the location of living houses and outbuildings inside the fenced courtyard (Reutti 1994). The *Axial-typ* or *Achsenhof* means the regular order, the axial classification of farm buildings. In the case of the so-called *Streuhof-typ*, the buildings are placed scattered, without any geometric order in the yard. These categories can not be applied on the villas of Southern Lusitania based on our present knowledge. However, the encircling walls, the fences can be found with some villas, clearly separating the *pars urbana* and pars *rustica* from each other.

Probably, ornamental gardens belonged to the pars urbana, too; the roots of them go back to the cities, like at the scheme of *domus* (Castiglione 1971, 1973, 1979, Jashemski 1979). The urban gardens (*hortus*, *viridarium*) were small, apart from a few rich examples, and they had decorative functions primarily, with useful spices and herbs as well. The appearance of aqueducts in houses caused changes in the equipment of the reception rooms (*atrium*) and inner gardens (*peristylium*): the rainwater catchment basins were replaced by marble, with fountains and statues decorating the basins. The excavated houses of the Roman town Conimbriga show the varied formations of these pools. The water arrived to the city in well-built aqueducts and it had a large-scale use: e.g., as the water of geometric pool-systems with planting islands; the small fountains had favourable effect on the climate (Alarcão 2000, Correia 2008, Moutinho Alarcão 1997). Dining spaces were often placed in the gardens, too; a triclinium was excavated in the garden of the *villa* at Cerro da Vila (Marques 1992).

The economic life and agriculture of Lusitania

The Celts started to use more advanced agricultural and cultivation techniques than the natives had then in Portugal. The population living here was extremely complex at the beginning of the Roman conquest, but the Roman colonization reduced the ethnic differences of the peninsula. The

Romans recognized the countryside, the natural features during the wars against Carthage, and they used these potentials after the conquest of this area (Saraiva 2010). Wheat, grape and olive were the most suitable for the local climate and soil conditions. The production of these plants came on such an advanced level with the involvement of the labour of indigenous people in the Roman period that the products could be exported, too (Thompson 1918, Saraiva 2010).

The first *villas* were owned by veterans and Italian immigrants initially, and they built on the most fertile areas: these fields were made productive with slave labour. The Roman plough replaced the previuos one south from the Tagus; however, the natives retain their tools in the north (Saraiva 2010). The process of development of the Hispanian agriculture and the exploitation of marine resources started at the foundation of Lusitania province, and it peaked in a few decades. The centres of this process were the villas presented here (e.g., Abicada, Cerro da Vila): these farms followed each other along the coast. The first villa foundations in Southern Lusitania started in the second half of the 1$^{\text{st}}$ century. The new constructions, alterations and technical ameliorations reflect the continuous development and growing wealth in the second half of the 3$^{\text{rd}}$ century; they are the witnesses of the expansion of production capacity and the economic success of the owners. Inter alia, the oil presses in Milreu and the large workshop in Cerro da Vila were established at that time (Teichner 2005, Moutinho Alarcão 1997). The economic crisis striking the Empire in the 3$^{\text{rd}}$ century did not reflect its impact in Southern Lusitania: abandoning some of the villas located here was caused rather by natural events (for example, a big flood at Cerro da Vila; see Teichner 2005).

Results and discussions. The villas of southern Lusitania (Algarve)

Becoming acquainted with Roman agriculture is essential to the examination of the regional and cultural context of the *villas*. The works of the ancient authors (*auctor*), the wall paintings and mosaic pavements (on Lusitanian mosaics, see Oliveira 2010) as well as the fine arts provide a picture of the country life and agriculture. Besides these, the aerial photographs can also show possession structures and cultivation forms in rare cases.

The most prominent agricultural authors were Cato, Varro, Columella, Pliny the Elder and Pliny the Younger, and Palladius: they wrote about the estates, cultivation and management forms and processes, about the cultivated plant species and tools as well. The ancient authors gave useful pieces of advice for the owners of the farms: they wrote about the selection of the right location for building a *villa*, about the right placement of living houses and outbuildings on the farms. It was beneficial for the *villa* if it had a water source inside of the fenced area or nearby the farm, if it had a good kitchen and a cellar according to the size of the estate. Cato emphasized the observation of the neighbours' welfare connected to the aspects of purchasing an estate: the climate is certainly favourable and the soil is good if the neighbours live well (Cato I. 2.). The most important aspects for an ideal estate are: "if possible, it should lie at the foot of a mountain and face south; the situation should be healthful, there should be a good supply of labourers, it should be well watered, and near it there should be a flourishing town, or the sea, or a navigable stream, or a good and much travelled road" (Cato I. 3.). The size of the ideal estate is of 100 *iugerum* (about 25 acres; see Heimberg 1977). Cato emphasized the importance of a well-built *pars rustica*: oil and/or wine cellars (*cella olearia* and *cella vinaria*) with barrels and runlets (*dolia*) and good presses (*torcularia*) included into the equipment of estates, in addition to the buildings.

Varro's opinion was that the aim of agriculture is the pursuit of profit and pleasure (*ad utilitatem et voluptatem*). However, he emphasized the importance of the beauty of the land and estate, which is advantageous at sale (Varro 1.4.1-2.). A farm is profitable when the costs of transportation of the produced goods are favourable and if the delivery of the materials needed by the *villa* is low-cost (Varro 1.16.3): "if there are towns or villages in the neighbourhood, or even well-furnished lands and farmsteads of rich owners, from which you can purchase at a reasonable price what you need for the farm, and to which you can sell your surplus, such as props, or poles, or reeds, the farm will be more profitable than if they must be fetched from a distance; sometimes, in fact, more so than if you can supply them yourself by raising them on your own place." Nevertheless, high-quality roads and navigable rivers for the transport of the products have to be available as well (Varro 1.16.6). In Columella's opinion, the mixed intensive farming was the best form of cultivation and management, where the cereal, the olive trees and vineyards, the livestock and grazing are in about same proportions.

The cultivation of grapes and olives, the production of wine and oil were the most profitable sectors in the Roman agriculture. The authors had divided opinions about the ideal orientation of the vineyards (*vinea*): Virgil suggested western, Columella eastern or southern aspects, but the most appropriate place has to be experienced in Pliny's point of view, the selection of the ideal area depends on the location and the potential of the possession (Firnigl 2012). The climate of Lusitania was perfect for cultivating olives and especially grapes, as mentioned above.

Nearly 40% of Hispanian villas were built on alluvial soils, what is particularly favourable for wheat cultivation, stock-raising and home gardens: this is mainly due the fact that the alluvial soils are along the rivers, and the Romans settled close to the rivers (see Gorges 1979, Edmondson 1981). Portugal is primarily an agricultural country, three-quarters of its area is fertile (one-third of it is arable land and one-third is covered with forests and wooded areas; this remained also in the 20^{th} century; see Thompson 1918). However, the maritime trade had primary importance in Southern Lusitania (Teichner 2004).

The determined *villas* in five municipalities (*concelho*) of the region Algarve are examined here, dealing especially with the excavated sites. Six villas are identified in Portimão (e.g., Bairro da Boavista, Abicada), five in Loulé (for example, Milreu, Cerro da Vila), one in Lagoa, six in Silves, while Roman villas are not known in Albufeira (Figure 5).

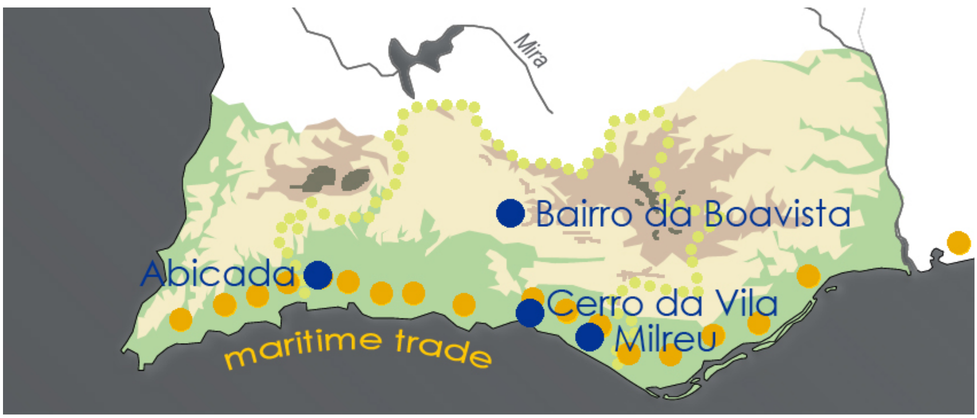

Figure 5: The presented villas of Southern Lusitania with other settlements, necropolis and the sites of maritime trade (after Marques 1992 and Moutinho Alarcão 1997, p. 39.)

The main building of the *villa* of Bairro da Boavista (Portimão) has mosaic pavement and it is located approximately 500 m far from the River Barranco do Poco. A mill and a large twin-tank are known in its pars rustica (Marques 1992, Moutinho Alarcão 1997). The villa of Abicada (Mexilhoeira Grande) was lay close to the ancient port city of Portus Hannibalis: its area is ca.

250 m far from the mouth of two rivers, exposed to the Atlantic winds. The main building of the pars urbana was made on the classical scheme of villa maritime, probably in the 2nd–3rd centuries. So far, little information is available about the *pars rustica*: fishery products (for instance, garum, one of the main materials of Roman cuisine; see Apicius 1996) were prepared here (Marques 1992, Keay 2003, Teichner 2005).

The *villa* of Milreu (Estoi) was built in the hinterland of the port city of Ossonoba, beside the Faro-Beja road, on a western slope at Rio Seco (Figure 4). The villa has an antecedent from the early Empire, which was rebuilt as a centre of a large estate at the end of the 1st century. A rich main building with mosaic pavement and large central courtyard (*peristylium*), and a bath (*balnea*) were in the *pars urbana*. Workshops, mills, water reservoirs and halls – probably for the large-scale production of olive oil and wine – were built in the pars rustica. In addition, two mausoleums and a cult building from the 4th century (*nymphaeum*) are also known here, which remained in use even after the Roman era (Teichner 2005).

The *villa* of Cerro da Vila (Quarteira) was built approximately 12 km west-northwest from Osoonoba. A bay was here in the Roman period: this natural harbour was favourable for the settling (the siltation of the northwestern part of this bay and the isolation from the ocean caused the depopulation of many surrounding settlements and *villas*, including Marmeleiros from the 1st century, neighbouring Cerro da Vila; see Marques 1992, Teichner 2005). The proximity of the ocean (fishing and maritime trade) gave the richness of Cerro da Vila and its continuous development. The settling time of the *villa* is probably the first half of the 1st century, thanks to the territorial reorganization related to Augustus's province establishment. A main building with *peristylium* and *thermae* stood in the centre of the *pars urbana*. The regulated water supply was solved at high costs: the water was led from the dam of Vale de Tesnado from 1600 m distance (Moutinho Alarcão 1997 and see Figure 3). The water was essential for the factories for seafood products in the *pars rustica* (*fabricae*, the factories at Cerro da Vila – from the beginning of the 2nd century – were the biggest commercial facilities of Lusitania known today), and for a *therma*. Several residential buildings are also known in the pars rustica, what indicates a large number of workers. Two mausoleums and a necropolis belonged to the *villa* as well (Marques 1992, Teichner 2005).

Streams or smaller rivers pass through the 150–500 m large environment at all of the 18 examined villas. These settlements were built in the lowest elevation zones, at the average height of 50–100 m, mostly on southern or western slopes (none of the *villas* are situated over 400 m height; this feature can be found in other provinces, too, e.g., in Dacia and Pannonia; see Wanner 2010, Firnigl 2012).

Conclusions

The Romans fell into deep economic crisis at the beginning of the 5th century. Their political and administrative system was torn apart by the barbarian tribes invading from Eastern Europe: neither the Romans nor the Roman-ally Visigoths were able to repel the attack of the Alans, Vandals and Suebis. The shortage of slaves, the basics of economy, and the stagnant export, then the expansion of the barbarian tribes led to the final decline and disintegration of the province (Saraiva 2010). However, the *villa* system survived, several buildings were inhabited even in the 8th century and in the Islamic period, and the characteristic size of estates was clearly visible in the 20th century, too.

References

[1] Alarcão, J. de (1988), Roman Portugal. Aris & Phillips, Warminster.

[2] Apicius, M. G. (1996), Szakácskönyv a római korból [De re coquinaria]. Enciklopédia Kiadó, Budapest.

[3] B. Thomas, E. (1961), Rómaikori villák a Balatonvidéken [Roman Villas on the Balaton Highland]. Képzőművészeti Alap Kiadóvállalata, Budapest.

[4] Bechert, T. (2005), Hof oder Halle? Anmerkungen zur Überdachung des zentralen Innenbereichs kaiserzeitlicher Risalitvillen. Balácai Közlemények IX.: 165–176.

[5] Butzer, K. W. et al. (1985), Irrigation Agrosystems in Eastern Spain: Roman or Islamic Origins? Annals of the Association of American Geographers 75/4: 479–509.

[6] Castiglione L. (1971), Római művészet [Roman Art]. Corvina, Budapest.

[7] Castiglione L. (1973), Pompeji. Corvina, Budapest.

[8] Castiglione L. (1979), Pompeji. Herculaneum. Gondolat Könyvkiadó-Magyar Helikon Könyvkiadó, Budapest.

[9] Cato (2003) De agri cultura In: Borhy, L. (ed.): Római történelem. Szöveggyűjtemény. Osiris Kiadó Kft., Budapest

[10] Edmondson, J. C. (1981), Review - Gorges, J.-G.: Les Villas Hispano-Romaines: Inventaire et Problématique Archéologiques/Paris, 1979/. The Journal of Roman Studies 71: 217–218.

[11] Edmondson, J. C. (1989), Mining in the Later Roman Empire and beyond: Continuity or Disruption? The Journal of Roman Studies 79: 84–102.

[12] Firnigl, A. (2012), Római kori villák történeti környezetének vizsgálata a Balaton-felvidéken [The Examination of the Historical Environment of Roman Villas on the Balaton Highland]. PhD Dissertation, typescript, Corvinus University of Budapest.

[13] Gorges, J-G. (1979), Les Villas Hispano-Romaines. Inventaire et problématique archéologiques E. de Boccard, Paris.

[14] Heimberg, U. (1977), Römische Landvermessung. Gesellschaft für Vor- und Frühgeschichte in Württemberg und Hohenzollern, Stuttgart.

[15] Jashemski, W. F. (1979), The Gardens of Pompeii, Herculaneum and the Villas destroyed by Vesuvius. Caratzas Brothers, New Rochelle.

[16] K. Palágyi, S. (1994), Őskor, római kor, népvándorlás kora. In: Veress D., Cs. (ed.): Nemesvámos története - A község története az ősidőktől napjainkig [History of Nemesvámos from the ancient times to the present days]. Gubicza Ferenc, Veszprém, pp. 7–47.

[17] Keay, S. (2003), Recent Archaeological Work in Roman Iberia (1990–2002). The Journal of Roman Studies 93: 146–211.

[18] Marques, T. (ed.) (1992), Carta arqueológica de Portugal: Concelhos de Portmao, Lagoa, Albufeira, Loulé e Sao Bras de Alportel. IPPAR, Lisboa.

[19] Marzano, A. (2007), Roman Villas in Central Italy. A Social and Economic History. Columbia Studies in the Classical Tradition, vol. 30.

[20] McKay, A. G. (1980), Römische Häuser und Paläste. Raggi Verlag, Feldmeilen.

[21] Moutinho Alarcão, A. (ed.) (1997), Portugal Romano: A exploraçao dos recursos naturais. S. n., s. l.

[22] Mócsy, A. (1974), Pannonia a korai császárság korában [Pannonia in the early Roman Empire]. Akadémiai Kiadó, Budapest.

[23] Mócsy, A., Fitz, J. (eds.) (1990), Pannonia régészeti kézikönyve [Archaeological Guide of Pannonia]. Akadémiai Kiadó, Budapest.

[24] Mócsy, A. (1995), Pannonia városai, falvai és villái a II. század közepén. In: Stirling, J. (ed.): Szöveggyűjtemény a kertépítészet történetének tanulmányozásához – I. Klasszikus ókor, I.1. Róma. Kertészeti és Élelmiszeripari Egyetem, Budapest, pp. 157–161.

[25] Oliveira, C. Fernandes de (2010), Mosaicos romanos de Portugalo o Algarve oriental. PhD Dissertation, Coimbra.

[26] Reutti, F. (1994), Typologie der Grundrisse römischer Villen. Balácai Közlemények III.: 200–205.

[27] Saraiva, J. H. (2010), Portugália rövid története [Short History of Portugal]. Equinter, Budapest.

[28] Teichner, F. (2004), Purpur, Olivenül und Fischsaucen als Exportartikel. Wirtschaftsarchäologische Untersuchungen am.

[29] Teichner, F. (2005), Neue Forschungen zur Ländlichen Besiedlung im Süden der römischen Provinz Lusitanien. Balácai Közlemények IX.: 117–132.

[30] Thompson, W. (1918), Portugal: The Country and the People. Geographical Review 6/2: 147–155.

[31] Varro (2003) Rerum rusticarum libri tres. In: Borhy, L. (ed.): Római történelem. Szöveggyűjtemény. Osiris Kiadó Kft., Budapest and after www.rbedrosian.com

[32] Vitruvius (1988), Tíz könyv az építészetről [De architectura]. Képzőművészeti Kiadó, Budapest.

[33] Wanner, R. (2010), Forts, fields and towns: Communities in Northwest Transylvania from the first century BC to the fifth century AD. PhD Dissertation, typescript, University of Leicester.

[34] White, K. D. (1966), H. Dohr, Die italischen Gutshöfe nach den Schriften Catos und Varros. Review. The Journal of Roman Studies 56: 249–250.

Acta Universitatis Sapientiae
Agriculture and Environment, **5**, (2013) 56–68

Evaluation of freezing pre-treatments for the lyophilisation of apple

Tamás ANTAL[1]
email: antalt@nyf.hu

László SIKOLYA[2]
email: sikolya@nyf.hu

Benedek KEREKES[1]
email: kerekesb@nyf.hu

[1]College of Nyíregyháza,
Department of Vehicle and Agricultural Engineering
[2]College of Nyíregyháza,
Department of Transportation and Infotechnology

Abstract. The effect of freezing rate on the quality of dried Jonagold and Idared (Malus domestica Borkh.) was studied. Apple slices underwent various pre-treatments, i.e. freezing in household freezer (freezing speed/rate: 0,5°C/min), contact plate freezing (2°C/min) and vacuum-freezing (3°C/min). The quality of the freeze-dried product was then evaluated in terms of water activity (aw), hardness, color and rehydration. The texture and color experiments were carried out with texture analyser and colorimeter. The aw of apple slices was measured by aw apparatus.

It was found that drying time was influenced by freezing rate. The freezing in household freezer (slow freezing rate) significantly reduces the duration of the freeze-drying process and consequently the process costs. The slow freezing rate allows the growth of large ice crystals at the beginning of the freeze-drying process; this fact should consequently lead to larger pores and injured cell walls and thus to shorter freeze-drying time.

Quality of the freezing in household freezer product was assessed as higher than the quality of the other freezing pre-treated material. Slow freezing rate resulted softer texture and higher rehydration capacity than that of other pre-treated samples. In all cases, slow freezing speed lead to lower final moisture content, total color difference and water activity.

Freeze-dried samples prepared with higher freezing rates (3°C/min) were the most white in color because small pores, originated by sublimation of small ice crystals formed by fast freezing.

Keywords: lyophilisation, vacuum-freezing, rehydration

1 Introduction

Freeze-drying is a dehydrating process for the long-term preservation of heat sensitive food and other biological materials based on the phenomena of sublimation. The freeze-drying process consists of three stages (1) freezing of the material, (2) a first drying stage, where sublimation of the frozen free water occurs, and (3) a second drying stage, where the bound frozen water is eliminated by desorption (Reyes et al. 2010). Freezing is a previous operation in freeze-drying in which they develop the structure and therefore predetermine the properties of dried material (Ceballos et al. 2012). Freezing plays an important role in material preservation and dehydration (Jackel et al. 2008). Freezing method, freezing temperature and freezing time are very important parameters in the initial stage (Wang et al. 2012). In terms of the drying kinetics, physical pre-treatment such as freezing, changes the physical properties of the sample cell structure, which in turn alters the rate of moisture removal (Eshtiaghi et al. 1994).

Generally, the faster the freezing rate, the smaller the ice crystals and hence, the longer the primary drying period. This has the advantage of giving a higher final product quality (Ghio et al. 2000). Araki et al. (2001) observed that a slower freezing rate, which leads to larger ice crystals, resulted in higher permeability in the frozen samples. Therefore, it is important to control the size of the ice crystals during freezing because it affects the quality of the freeze-dried product. It can influence and damage cell walls, porosity and hence drying kinetics as well as rehydration characteristics of freeze-dried products.

To reduce the freeze drying costs, one alternative is to evaluate an adequate freezing rate.

Apples are an important raw material for many food products and apple plantations are cultivated all over the world in many countries. The apples are consumed either fresh or in the form of various processed products such as juice, jam and marmalade, dried apples, etc. (Doymaz 2009).

The research aimed to improve the quality of freeze-dried apple by various freezing rate (0.5, 2 and 3°C/min). The effect of freezing pre-treatments on both the drying time and some quality attributes of dried apple slices, such as moisture content, water activity, color, texture and rehydration were investigated.

2 Material and Methods

Material

Fresh apples (var. Jonagold and Idared) (Malus domestica Borkh.) were purchased from a local market and stored at 5°C. Prior to an experiment, apple was washed with tap water, peeled and sliced to a thickness of 5 mm using a hand slicer. The initial moisture content of Jonagold and Idared apple slices were 86,5%, wb. (6,4 kg water/kg dry matter, db.) and 87,6%, wb. (7,064 kg water/kg dry matter, db.). The moisture content – before and after treatment – was determined by gravimetric method. At regular time intervals during the processes, samples were taken out and dried in the oven for 3–4 hrs at 105°C until a constant weight was achieved. Weighing was performed using a digital balance, and then moisture content (wet basis (wb.), dry basis (db.)) was calculated. The tests were performed in triplicate. All apple samples were randomly divided into groups for each treatment.

Freezing pre-treatments

The freezing of the apple samples, achieved when using three different freezing equipment, where: (1) freezing in household freezer (freezing rate: 0,5°C/min, cooling down to −25°C), (2) freezing in contact plate freezer (freezing rate: 2°C/min, to −25°C) and (3) freezing in chamber of vacuum freezer (freezing rate: 3°C/min, to −25°C). Two freezing rates (medium and slow) were used to freeze apple slices in order to determine changes in apple quality characteristics. The thermocouples were inserted in the top, in the middle and in the bottom of the sample in order to obtain a good and reproducible result. After pre-treatments, the samples were dried in the vacuum freeze dryer. In every drying experiment, about 200 g apple slices were pre-treated. All measurements were performed in triplicate.

Freeze drying

The experiment was conducted using a laboratory scale freeze dryer (FT33, Armfield Ltd, Ringwood, England) equipped with a computerized data acquisition system. The moisture loss is recorded during drying by a specially developed weighing unit. The weighing unit consists of a load (model PAB-01, 500±0.1 g) and registering instrument (model ES-138, Emalog, Budapest, Hungary). The product was dried for a period of 22–24 hrs at 45–82 Pa with the heating plate kept at 18°C. The condenser temperature was kept at −50 to −55°C. The experiments were replicated three times and the average of the moisture content at each value was used.

Water activity

Water activity (aw) is defined as the ratio of the vapour pressure of water in the food to the vapour pressure of pure water at the same temperature (McLaughlin and Magee 1998). Water activity indicates how tightly the water is bound in the food from a structural and chemical point of view, and hence it describes the availability of the water to participate in chemical and biochemical reactions. The water activity scale extends from 0 (bone dry) to 1.0 (pure water) but most foods have a water activity level in the range of 0.2 for very dry foods to 0.99 for moist fresh foods.

The water activity of apple slices was measured at 25°C, using a temperature-controlled Novasina Labmaster (model CH-8853, Novasina AG, Switzerland). The determination was performed in triplicate.

Color

Color is an important quality attribute in a food to most consumers. The color of apple slices was measured with ColorLite sph900 spectrophotometer (ColorLite GmbH, Germany) before and after drying. The color meter was calibrated against a standard calibration plate of a white surface and set to CIE $L*a*b*$. The color parameters ($L*, a*, b*$) were measured at 5 different points and the average was calculated for each sample. The color brightness coordinate $L*$ measures the whiteness value of a color and ranges from black at 0 to white at 100. The chromaticity coordinate $a*$ measures red when positive and green when negative, and chromaticity coordinate $b*$ measures yellow when positive and blue when negative. In addition, the total color difference (ΔE) was calculated using Eq. (1), where L_0, a_0 and b_0 are the control values for fresh apple.

$$\Delta E = \sqrt{(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2} \tag{1}$$

Textural characteristics

Measurements of the texture of apple slices were carried out using a texture analyser (CT3-4500, Brookfield Engineering Laboratories, Middleboro, USA). The parameters of the test were as follows: 4.5 kg force load cell, 1 mm/s test speed, 10 g trigger force. The samples were placed on a hollow planar base, and afterwards were compressed with 4 mm cylindrical probe. The depth of penetration was 2 mm. The maximal compressive force recorded during the test was defined as hardness. The average value of maximum firmness for each treatment was then calculated and the results were expressed as Newtons.

Rehydration

Rehydration indicates the physical and chemical changes during freezing and drying as influenced by processing conditions, sample pre-treatment and composition (Feng and Tang 1998). The rehydration test was done by measuring the weight gain of dehydrated samples (ca. 2g) that were immersed in 250 ml of distilled water at a constant temperature of 25°C. Samples were withdrawn at 1, 5, 15, 30, 60 and 90 min, drained, wrapped in absorbent tissue to remove surface water, and weighed on an analytical balance (JKH-500, Jadever, Taiwan). The rehydration rate (RR) was calculated from the sample weight before and after rehydration. The rehydration measurements were made in triplicate for all the samples and average values were reported.

Statistical analysis

All data were subjected to the analysis of variance (ANOVA) using SPSS software and were presented as mean values with standard deviations. Differences between mean values were established using Duncan's multiple range tests at a confidence level of 95% ($p \leq 0,05$). All experiments were performed in triplicate.

3 Results and discussion

Influence of cooling pre-treatments on drying time

Curves of product temperature versus freezing–drying time for pre-treated apple samples are shown in Fig. 1. According to the results in Fig. 1, freezing pre-treatment is an important parameter that affects drying time. Freezing time for fresh apple slices varied from 13, 19 to 70 min, when temperature in the core of samples decreased from 14 to -25°C (vertical red line).

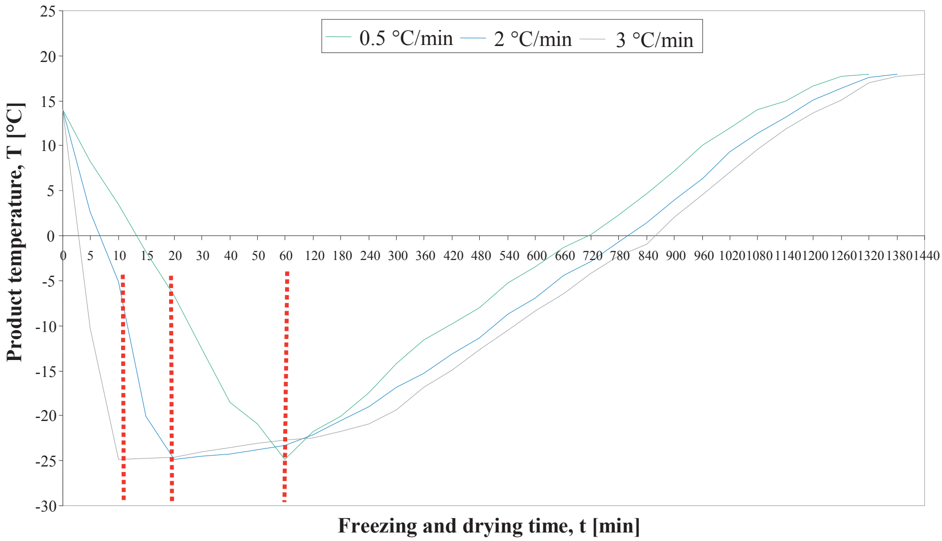

Figure 1: Temperature profile within Jonagold slices during freezing and sublimation process

The overall freezing and drying process, depicted in Fig. 1, starts with a freezing step (the end of this running is labeled with a vertical red line), followed by the first drying step, in which mainly the frozen water is removed by sublimation. A second drying step follows, in which bound water (unfrozen) is removed by desorption. In order to reduce the drying time in the first drying stage (sublimation), the size of ice crystals should be large (green line). Increase in drying rate is observed when fast freezing is used, probably due to the generation of smaller crystals in the first case, which is adverse for drying time (grey line).

Finally, it can be observed that the freezing rate of samples affects the drying kinetics. Nail and Gatlin (1993) affirmed that the greatest resistance of vapour transport occurs across the dried product layer, where water molecules have to pass the pores and channels, depending on the size of ice crystals. Thus, the slow freezing treatment increases the ice crystal mean size, improving the water vapour mass transfer, giving higher permeability of the dried layer and consequently reducing the primary drying times.

We did not plot the running of the temperature curves of Idared apple because it is very similar to the Jonagold. Samples frozen in household freezer prior to freeze drying were found to have a significantly shorter drying time compared to pre-treated ones by vacuum-freezing samples. The drying time of frozen apple slices (0.5°C/min) was close to those of frozen in contact plate freezer (2 °C/min). In our present work, it was shown that slow freezing rate (0.5°C/min) reduced the drying time by 8.3 % and 4.1% more than faster freezing rate (3 °C/min) (Table 1.).

According to Rovero et al. (2001), increasing the porosity and the permeability of the dry matrix will increase the sublimation rate, so the slower freezing rate, the shorter drying time. The slow freezing tends to disrupt cell walls, allowing the moisture to escape more easily. We stated in a previous study that the cell size of the plant largely depended on the freezing rates. The higher the freezing rate, the smaller the cell size during the freezing step (Antal 2013). In Table 1, it can be noticed that the final water content was also favoured by lower freezing rates.

According to the results in Table 1, the freezing pre-treatments had an effect on the moisture content of apple slices. The least moisture content of apple was observed for freezing in household and vacuum freezer (Jonagold and Idared).

Table 1: Effect of freezing pre-treatments on the freeze drying time and final moisture content

Pre-treatments	Freezing in household freezer (0.5°C/min)	Freezing in contact plate freezer (2°C/min)	Freezing in vacuum freezer (3°C/min)
Drying time, t (h)			
Jonagold	22^a	23^{ab}	24^b
Idared	23^a	23^a	24^b
Final moisture content, w (%, wb) Final moisture content, M (kg water/kg dry matter, db)			
Jonagold	4.72 0.177	4.93 0.192	5.25 0.281
Idared	5.16 0.213	5.30 0.229	4.97 0.145

^{ab}different superscripts in the same row mean that the values are significantly different ($p \leq 0.05$)

Quality assessment of treated apple samples

The aw of apple samples at various pre-treatments can be observed in Table 2. The value of water activity was highest in raw material, followed by freezing in different equipments. The aw of slow-frozen apple slices was lowest compared to that of other pre-treatment (except of freezing in contact freezer at Jonagold).

Table 2: Effect of freezing rates on water activity (a_w) of apple slices

	Freezing in household freezer (0.5°C/min)	Freezing in contact plate freezer (2°C/min)	Freezing in vacuum freezer (3°C/min)	Raw material
	Water activity, a_w (dimensionless)			
Jonagold	0.464^{ab}	0.458^{a}	0.472^{b}	0.975^{c}
Idared	0.438^{a}	0.461^{b}	0.454^{b}	0.981^{c}

^{abc}different superscripts in the same row mean that the values are significantly different ($p \leq 0.05$)

The important feature to consider is that all values of water activity are below 0.5, which indicates that the growth of moulds, bacteria and yeast is not promoted and enzymatic reactions are not likely to occur.

Table 3 shows the evaluated mechanical parameters obtained from the compression test. The slow freezing rate (household freezer) provided the texture of Jonagold apple similar to those of the frozen in contact freezer.

Table 3: Effect of freezing pre-treatments on the hardness of apples

	Freezing in household freezer (0.5°C/min)	Freezing in contact plate freezer (2°C/min)	Freezing in vacuum freezer (3°C/min)	Raw material
	Hardness – Compressive force, $F_c(N)$			
Jonagold	5.677^{a}	5.591^{a}	6.127^{b}	8.221^{c}
Idared	4.344^{a}	5.481^{b}	5.755^{c}	7.436^{d}

^{abc}different superscripts in the same row mean that the values are significantly different ($p \leq 0.05$)

The hardness values of Idared apple obtained for freezing in household freezer were the lowest of all samples. Slightly lower values were measured in the Jonagold slices between household and vacuum freezing. From our investigations, it is clear that the vacuum freezer method was the worst. The firmness of frozen and dried apple tissue strongly depends on physical (cell wall) and chemical changes due to the freezing speed.

Table 4 shows that the treatment with slow freezing has better color parameters (L and ΔE) than those of other pre-treatments. When the samples were frozen in household freezer, the lightness (L^*) and color differences (ΔE) of the apple were significantly lower than those undergone faster freezing. The rate of freezing has a marked effect in the brightness of the dried samples: quick frozen apple slices maintained a whiter color than those frozen more slowly (Ceballos et al. 2012). Nevertheless, the effect of higher freezing rate on the redness (a^*) and yellowness (b^*) was not significantly different among pre-treated samples.

Table 4: Effect of freezing rates on the color attributes of apple samples

Pre-treatment	Freezing in household freezer (0.5 °C/min)				Freezing in contact plate freezer (2 °C/min)				Freezing in vacuum freezer (3 °C/min)			
Color value	L^*	a^*	b^*	ΔE	L^*	a^*	b^*	ΔE	L^*	a^*	b^*	ΔE
Jonagold	69.45^a	2.34^a	19.23^a	9.7^a	71.92^b	2.47^a	21.11^{ab}	12.06^b	76.55^c	2.51^{ab}	20.28^a	14.38^c
Idared	76.41^a	1.51^a	13.67^a	7.33^a	81.77^b	1.29^a	14.59^a	11.3^b	83.34^{bc}	1.18^a	15.41^{ab}	16.84^c

^{abc}different superscripts in the same row mean that the values are significantly different ($p \leq 0.05$)

The higher L values represented decreasing in the degree of browning in frozen and dried samples, probably due to the fact that freezing process provoked a decrease in polyphenol oxidase activity responsible for browning. Vacuum freeze treatment showed the largest increase in L value from 65.54 to 76.55 and from 73.81 to 83.34 at Jonagold and Idared samples.

It was stated that the permeability of the rehydrated cell wall depended on the applied cooling rate. Rehydration curves of pre-treated apple slices at different rehydration time are shown in Fig 2. It can be seen that the rehydration rate of pre-treated Idared and Jonagold samples with slow freezing (0.5°C/min) resulted in the highest rehydration, compared to faster frozen samples. This might be due to a smaller pore size left by faster freezing (2 and 3°C/min), which is filled up slowly with a distilled water. It was stated that the permeability of the rehydrated cell wall depended on the applied cooling rate. Rehydration curves of pre-treated apple slices at different rehydration time are shown in Fig 2. It can be seen that the rehydration rate of pre-treated Idared and Jonagold samples with slow freezing (0.5°C/min) resulted in the highest rehydration, compared to faster frozen samples. This might be due to a smaller pore size left by faster freezing (2 and 3°C/min), which is filled up slowly with a distilled water. The rehydration ratio increased within the initial period, but the rate gradually slowed. Similar results have been reported for red apple (Doymaz 2010).

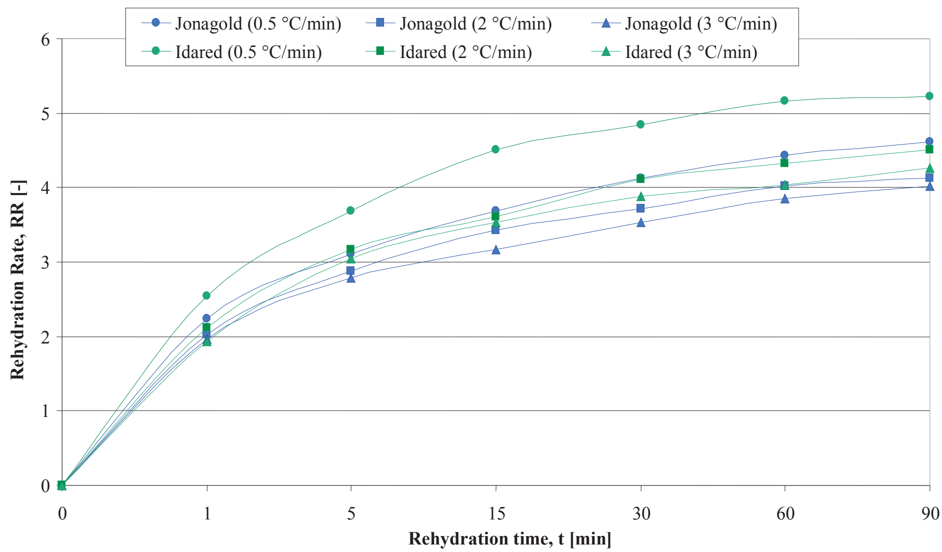

Figure 2: Rehydration rate of pre-treated apple samples in wetting medium at 25°C

The values of rehydration rate (RR) of frozen apple samples at 25°C and 90 min are listed in Table 5. From the results, it can be observed that the rehydration capacity in household freezer-cooled samples was higher than contact plate freezer- and vacuum freezer-frozen ones. There was no significant difference in the rehydration rate in the case of faster freezing rates (at Jonagold). The freeze-dried product has excellent rehydration capacity (RR is more than 4.00). It is due to the structural rigidity of the frozen product, which can prevent the collapse of the solid matrix remaining after drying (Beaudry et al. 2004).

Table 5: Effect of freezing rates on rehydration rate (RR) of apple cubes

Pre-treatments	Freezing in household freezer (0.5°C/min)	Freezing in contact plate freezer (2°C/min)	Freezing in vacuum freezer (3°C/min)
Rehydration rate, RR (-) at 90 min (soaking time, end of wetting process)			
Jonagold	4.62^a	4.13^b	4.02^b
Idared	5.23^a	4.51^b	4.27^c

^{abc}different superscripts in the same row mean that the values are significantly different ($p \leq 0.05$)

4 Conclusions

The effect of freezing pre-treatments on drying time and the quality of apple varieties using freeze drying was studied. It was observed that the lower cooling rate leads to shorter drying time of the samples (enhancing the moisture transfer). The drying time of Jonagold and Idared samples was reduced by 8.3 and 4.1% when using household freezer. It was observed that these values are negligible in terms of freeze drying. The freezing in household freezer caused lower final moisture content in dried samples. Samples pre-treated with slow freezing (0.5°C/min) have higher rehydration rates and better color (L^* and ΔE) compared to faster frozen (2 and 3°C/min) apple samples. Similarly, the water activity and firmness of slow frozen apple slices were superior than the pre-treated ones with other freezing methods.

Smaller crystals provide an advantage in frozen food products (microstructure), whereas larger crystals are useful for the running time of lyophilisation. Slow freezing reduces the duration of the freeze drying process and consequently the process costs.

Slow freezing rate causes severe changes in product microstructure and the final product presents broken surfaces, membrane rupture and cell wall degradation (Tregunno and Goff 1996). Pikal (2007) noted that a shorter freezing time is essential in reducing product degradation. It is therefore recommended that relatively fast freezing (up to 3°C/min) prior to drying should be conducted as a method for producing dried apple pieces.

Acknowledgements

Publishing of this journal is supported by the Institute for Research Programmes of the Sapientia University.

This research was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP 4.2.4. A/2-11-1-2012-0001 "National Excellence Program". (Tamás Antal)

This research was realized in the frames of TÁMOP 4.2.4. A/2-11-1-2012-0001 "National Excellence Program-Elaborating and operating an inland student and researcher personal support system". The project was subsidized by the European Union and co-financed by the European Social Fund. (László Sikolya)

References

[1] Antal, T.: A fagyasztás, mint előkezelés hatása a szublimációs szárítás működési idejére és a végtermék minőségére. Élelmiszer Tudomány Technológia, 67 (2013/4).

[2] Araki, T., Sagara, Y., Abdullah, K., Tambunan, A. H.: Transport properties of cellular food materials undergoing freeze-drying. Drying Technology, 19 (2001), 297–312.

[3] Beaudry, C., Raghavan, G. S. V., Ratti, C., Rennie, T. J.: Effect of four drying methods on the quality of osmotically dehydrated cranberries. Drying Technology, 22 (2004/3), 521–539.

[4] Ceballos, A. M., Giraldo, G. I., Orrego, C. E.: Effect of freezing rate on quality parameters of freeze dried soursop fruit pulp. Journal of Food Engineering, 111 (2012), 360–365.

[5] Doymaz, I.: An experimental study on drying of green apples. Drying Technology, 27 (2009), 478–485.

[6] Doymaz, I.: Effect of citric acid and blanching pre-treatments on drying and rehydration of Amasya red apples. Food and Bioproducts Processing, 88 (2010), 124–132.

[7] Eshtiaghi, M. N., Stute, R., Knorr, D.: High-pressure and freezing pretreatment effects on drying, rehydration, texture and color of green beans, carrots and potatoes. Journal of Food Science, 59 (1994), 1168–1170.

[8] Feng, H., Tang, J.: Microwave finish drying of diced apples in a spouted bed. Journal of Food Science, 63 (1998), 679–683.

[9] Ghio, S., Barresi, A. A., Rovero, G.: A comparison of evaporate and conventional freezing prior to freeze-drying of fruits and vegetables. Institution of Chemical Engineering, 78 (2000), 187–192.

[10] Jackel, T., Dautel, K., Ternes, W.: Preserving functional properties of hen's egg yolk during freeze-drying. Journal of Food Engineering, 87 (2008), 522–526.

[11] McLaughlin, C. P., Magee, T. R. A.: The determination of sorption isotherm and isosteric heats of sorption for potatoes. Journal of Food Engineering, 35 (1998), 267–280.

[12] Nail, S., Gatlin, L.: Freeze drying: Principle and practice. In Avis, A. - Liebermann, A. - Lachann, I. (Ed.): Pharmaceutical dosage forms, Vol. 2. Marcel Dekker Inc., New York, 1993, 163–333.

[13] Pikal, M. J.: Freeze drying. In Swarbick, J. (Ed.): Encyclopedia of Pharmaceutical Technology. Informa Healthcare, New York, 2007, 1807–1832.

[14] Reyes, A., Bubnovich, V., Bustos, R., Vásquez, M., Vega, R., Scheuermann, E.: Comparative study of different process conditions of freeze drying of 'Murtilla' berry. Drying Technology, 28 (2010), 1416–1425.

[15] Rovero, G., Ghio, S., Barresi, A. A.: Development of a prototype capacitive balance for freeze-drying studies. Chemical Engineering Science, 56 (2001/11), 3575–3584.

[16] Tregunno, N. B., Goff, H. D.: Osmodehydrofreezing of apples: Structural and textural effects. Food Research International, 29 (1996), 471–479.

[17] Wang, Y., Zhang, M., Mujumdar, A. S., Mothibe, K. J.: Experimental investigation and mechanism analysis on microwave freeze drying of stem lettuce cubes in a circular conduit. Drying Technology, 30 (2012), 1377–1386.

Acta Universitatis Sapientiae
Agriculture and Environment, **5**, (2013) 69–79

Climate and pedological factors of slump development on a Transylvanian example

Csaba FAZAKAS
email: fazakascs@ms.sapientia.ro

Sapientia Hungarian University of Transylvania,
Faculty of Technical and Human Sciences, Tg. Mureş
Department of Horticulture

Abstract. Areas endangered by slump formation in the Măgherani Basin, part of the Nirajului Hills (Romania), are studied in the present paper. Time periods considered dangerous regarding the development of slumps and the degree of risk were determined. Pedological conditions of the model area are assessed based on field and laboratory measurements, self-constructed maps and literature data. These factors could have contributed to the formation of slump phenomena in the model area.

Keywords: slump, landslide susceptibility, rock oversaturation, Măgherani Basin

1 Introduction

Slumps – a special type of mass movement resembling the characteristic forms of the Transylvanian Plains (Câmpia Transilvaniei) – are also characteristic of the Măgherani Basin. The clay-marl-sand stratification of the basin and its piedmont basin character help the formation of these landslide types. Landslide sensitivity or landslide susceptibility means the probability in space and time of landslide formation [8] (Varnes 1984). The present paper assesses the susceptibility of slump formation regarding temporal and spatial differences. Temporal risk of the formation of slumps depends on climatic conditions. Correlation between precipitation and the formation of landslides is discussed in numerous publications: Chleborad et al. (2006), Coe et al. (2004), Zezere et al. (2005). Some of these describe specific cases, others analyse statistic relationships in order to establish correlation models or forecast models on the basis of precipitation quantity [1] [3] [9]. Ujvári and Buz (1973) recommend the including of the term “rock oversaturation” [7] in the gravitation movement calculations of slopes. Spatial susceptibility depends on the pedological conditions. Factors of spatial susceptibility are regarded to be the preconditions of landslides. Transformation of natural conditions by man (e.g., deforestation, cultivation) increases the probability of slump formation (Swanson & Dryness 1975, Chung et al. 1995, Montgomery et al. 1998) [6], [2], [5].

2 Material and methods

The research area is located in the administration area of Şilea Nirajului and Măgherani settlements, 38 km from the town of Târgu Mureş, in the foreground of the Gurghiu Mountains in the NE margin of the Transylvanian Basin (Fig 1).

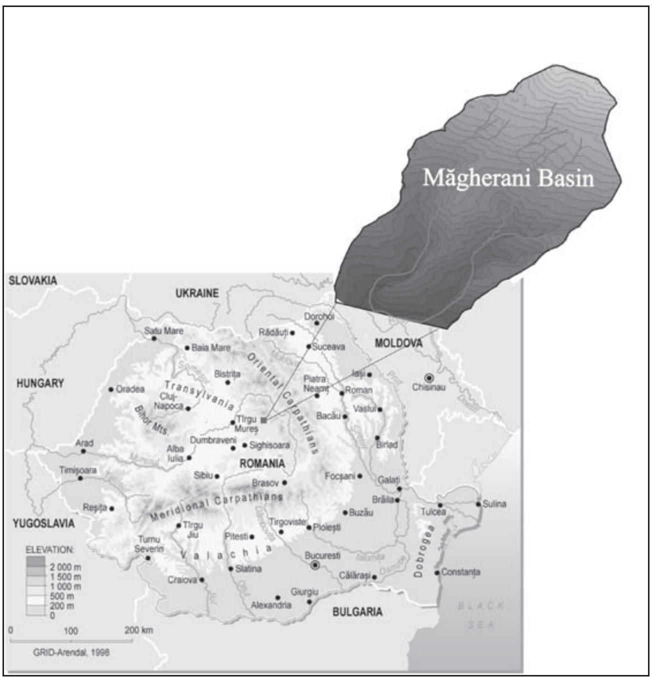

Figure 1: Location of the Măgherani Basin in Romania

This special type of mass movement, slump, is present in several areas of the Măgherani Basin, and extended areas are denuded due to its effects becoming unsuitable for cultivation.

Precipitation and temperature data concerning the year of the development of the slump at Şilea Nirajului (1999) and the two preceding years (1997 and 1998) were acquired as oversaturation triggering the slump could have taken place only in these years. Average monthly precipitation and monthly average temperature distributions are presented graphically (Fig. 2 and 3).

In order to study the pedological conditions of slump formation, the 1:10000 scaled cadastral and contour maps and the 1:25000 scaled military topographic maps were applied.

Soil conditions contributing to the formation of slumps were surveyed.

Field study of the soils was performed based on 15 dug soil profiles and 40 control drillings. The extension of the pedons was controlled based on analyses carried out using a Pürckhauer drilling rod. In the course of the pedological survey of the area, 8 soil types were identified. Samples were taken from each soil unit and analysed in laboratory.

Physical and chemical properties of the soils were analysed in the laboratory of the Mureş County Office for Pedology and Agrochemistry, applying the following methods: pH – potentiometric method; $CaCO_3$ determination – calcimeter with Scheibler's method; SB (basoid cation exchange capacity) – Kappen's method; SH (hydrogen ion exchange capacity) – Cernescu's method; grain-size distribution – Kacinski's method.

Special attention was paid to the physical soil properties determination (especially clay fraction) and to determine the presence and quantity of a material cementing the grains, as these properties determine the most the role of soils in the formation of slumps.

Mineralogical investigations were not possible; therefore, the published results of other scientists were studied.

3 Results and discussion

The graph presenting the yearly distribution of average temperatures (Fig. 2) reveals that the study area was characterized by high positive monthly average temperatures from March 1999 on, resulting in snowmelt, earlier than in other years.

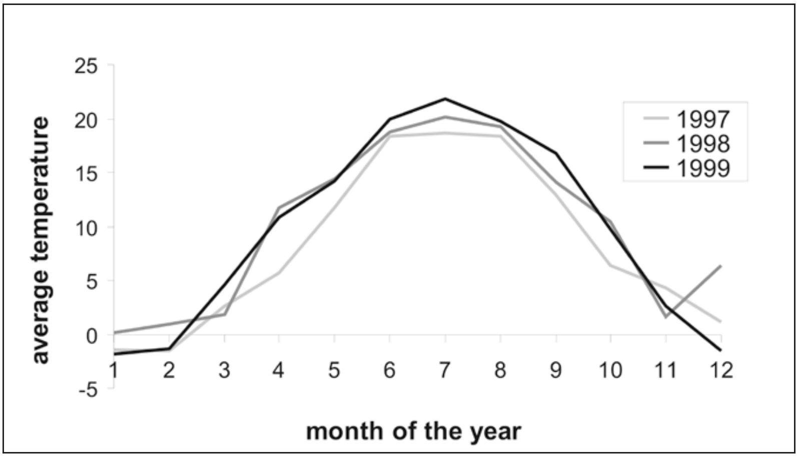

Figure 2: Annual distribution of average temperatures (1997, 1998, 1999)

On the graph showing the monthly precipitation values (Fig. 3), the amount of precipitation in the area in 1999 is compared to the amount of precipitation in the two previous years. The comparison reveals that although there was no record amount of precipitation in 1999, it exceeded 84 mm/m^2 – representing a great amount of precipitation in our region – in every month from April until July.

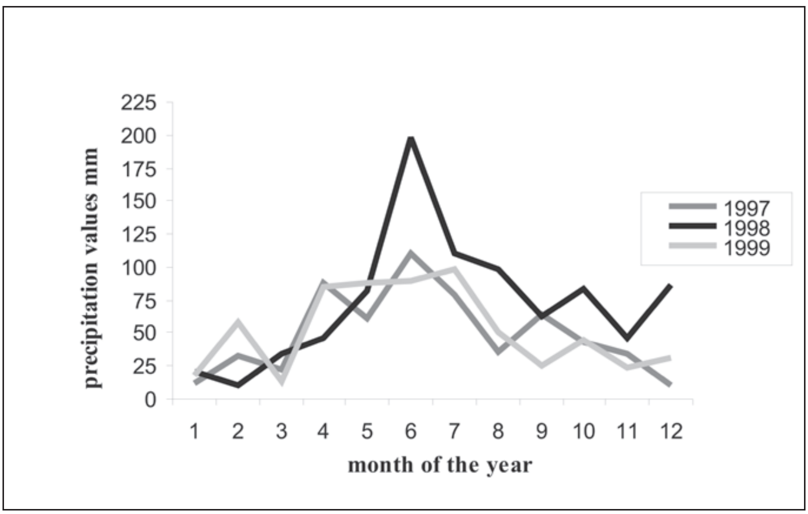

Figure 3: Distribution of the average monthly precipitation in the years 1997, 1998 and 1999

From May 1998, a period of vast precipitation started. Between May and October, and then in December as well, the amount of precipitation exceeded the average (Table 1) characteristic of our region for many years. The difference was 40–50 mm and even 120 mm in June. The second half of 1998 was characterized by precipitation excess.

Table 1: Comparison of the seasonal average precipitation of 1998 to the average of many years

Months	Average of many years (mm)	Average of 1998	Difference (mm)
VI-XII	408.5	685.1	+276.6
IX-XII	159.4	267.7	+108.3

Knowing the formation period of large slumps, the precipitation data, the period of snowmelt and the period of rock oversaturation, the graph of the risk of landslide formation was prepared (Fig. 4).

It was determined that the time period of lithological oversaturation lasts from January until May. Within this, the time period of the most frequent slope mobility lasts from March until May, i.e. it is connected to the time period following snowmelt. In June, which has the greatest precipitation, the mobility of the slopes is moderate while the time periods of the most stable slopes are August, October and November.

Water accumulated in the soil was unable to evaporate due to the low temperature in autumn, it was frozen in winter and then, as thawing in spring, it prepared the formation of the slump.

The Măgherani Basin is a small landscape unit that can be clearly defined geomorphologically. Its inner part, the 350–400 m high basin bottom is bordered from the valley of the Niraj river on the NW and W and from the valley of the Târnava Mică river on the S and SE by a mountain range rising over 500 m. On the NE and E, the border is formed by the Bichiş Mountain (1079 m). Streams running off the Bichiş Mountain exposed the monoclinal Pannonian strata in strike orientation and formed asymmetric valleys by landslides sliding down along the strata beds.

Ridges and ravines forming on the slopes developed over centuries and thousands of years forming wide valleys eventually. These processes are going on even today in a landscape where the ratio of slopes steeper than 5% gives more than 90% of the entire land; thus, current slope processes give a strong mark on land use, especially from agricultural point of view.

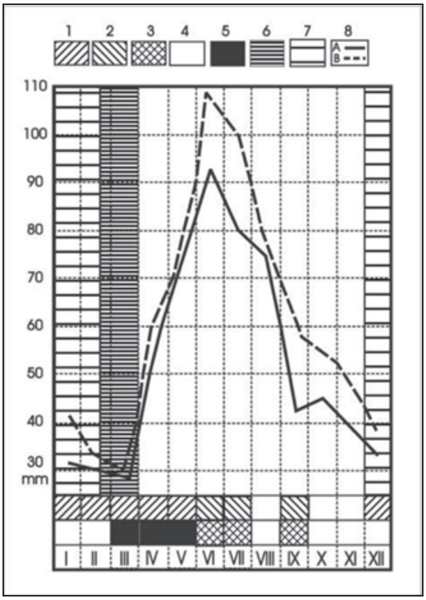

Figure 4: Graph showing the periods considered dangerous regarding slump formation and the degree of risk: 1 - time period of lithological oversaturation, 2 - time period of random, temporary oversaturation, 3 - time period of the moderate mobility inclination of slopes, 4 - time period of the maximum stability of slopes, 5 - time period of the greatest mobility inclination of slopes, 6 - time period of the final melt of snow cover, 7 - repeating melt period of snow cover, 8 - average precipitation amounts: A - in Târgu-Mureş and B - Măgherani

The Măgherani Basin cannot be regarded as geomorphologically stable due to the presence of several clay and marl layers in the Pannonian strata. The area is destined for slump-type mass movement development, geologically due to the following conditions:

- Clay and clay-marl strata have sheet-like thin stratification. The densely fractured character and loose internal structure of these (bedding planes form a poorly resistant surface) enable the filtration of precipitation water into deeper horizons.

- Thick-bedded slope sediments lay on the rocks forming the slopes with slope angle and petrographic discordance that has a significant role in the formation of landslides.

The geological conditions of the area determine the character and the temporal change of the relief pattern, the sloping conditions, the steepness, orientation and shape, etc. of the slopes, this way influencing the suitability of the land for the formation of mass movements. Geological conditions, however, also influence the physical-chemical properties of the soils. Grain-size distribution of the soils, their clay content and pH reflect the properties (determinative regarding slump formation) of the soil-forming sedimentary rocks.

Studying the soil cover of the area, it has become clear that the properties of the thick Pannonian slope sediments are favourable for the formation of slump-type mass movements. Based on the grain-size distribution of the soil, they are classified into the clayey silt – silt – silty clay texture types (Table 2).

Table 2: Soil types in the Măgherani Basin and their profile descriptions from grain-size distribution point of view

Nr.	Soil type	Horizon	Depth/cm	Texture			
				Coarse sand 2.0-0.2 mm	Fine sand 0.2-0.02 mm	Silt 0.02-0.002 mm	Clay < 0.002 mm
1	Stagnic Luvisols	Aa1	0-25	3.2	31.9	34.0	30.9
		Aa2	25-40	2.9	34.4	33.9	28.8
		Bt(B)g	45-55	4.3	27.4	31.0	36.2
		Btg	80-100	2.7	23.2	30.2	43.8
		Btg2	130-180	3.8	19.4	24.2	52.6
2	Distric Cambisols	A	5-30	6.0	40.6	29.7	24.6
		(B)	40-100	7.8	34.2	22.7	35.2
3	Colluvic Calcaric Fluvisols	Aa	0-20	4.4	36.5	32.4	26.8
		A'	20-35	5.8	36.2	29.5	28.4
		A"	50-80	5.0	33.2	27.9	33.7
4	Colluvic Humic Calcaric Fluvisols with mixed horizons	A	0-25	8.0	26.2	28.1	37.5
		AD	30-40	1.2	24.4	36.0	38.5
		D	70-100	0.4	20.6	38.4	40.8
5	Calcaric Gleysols	A(Go)	5-15	3.1	18.2	28.7	50.0
		A(Gr)	20-40	5.9	15.0	29.5	49.6
		D(Gr)	80-90	1.3	19.8	27.4	51.5
6	Stagnic Gleyic Faeoziems	Aa	5-20	3.4	24.7	29.2	42.7
		A'	35-55	0.6	21.9	32.0	45.5
		Bg	65-90	1.3	24.5	30.0	44.4
		CBg	130-160	2.6	33.0	32.2	32.2
7	Stagnic Regosol	Ba	0-18	13.1	52.2	14.3	20.4
		B	20-40	6.9	40.5	21.6	31.0
8	Calcaric regosol affected by landslides	C	0-22	3.1	28.3	31.3	37.3
		D	30-60	0.4	40.1	26.6	32.9

In the marginal parts of the areas characterized by slumps, the unmoved slope masses are variably fine-textured from the surface towards greater depth dominated by clay and silt-clay grain-size.

The soil-forming rock in the area of the basin is clay marl, which is clay containing a significant amount of lime. The active clay fraction (< 0.002 mm) is dominated by hydrated mica, mostly swelling illite/montmorillonite and beidellite-type clay minerals in both the weathered and unweathered rocks. Repeated swelling (in wetter periods) and shrinking (in dry periods) prepare the soils for sliding down the slopes.

Clay in-wash horizons marked with B(t) in the soils of the area cause poor water balance. As a result, a significant portion of the soils (43%) show pseudo-gleyic and gleyic characters. The hydromorphous character of the Stagnic Luvisols and the presence of the Stagnic Gleyic Faeoziems are the result of the soil-forming rock properties. Water filtrates very slowly into these soils. They are characterized by saturation with water filtrating parallel with the surface until late spring. Snowmelt following cold periods with excess precipitation and downpours in spring increases their slump-generating characters.

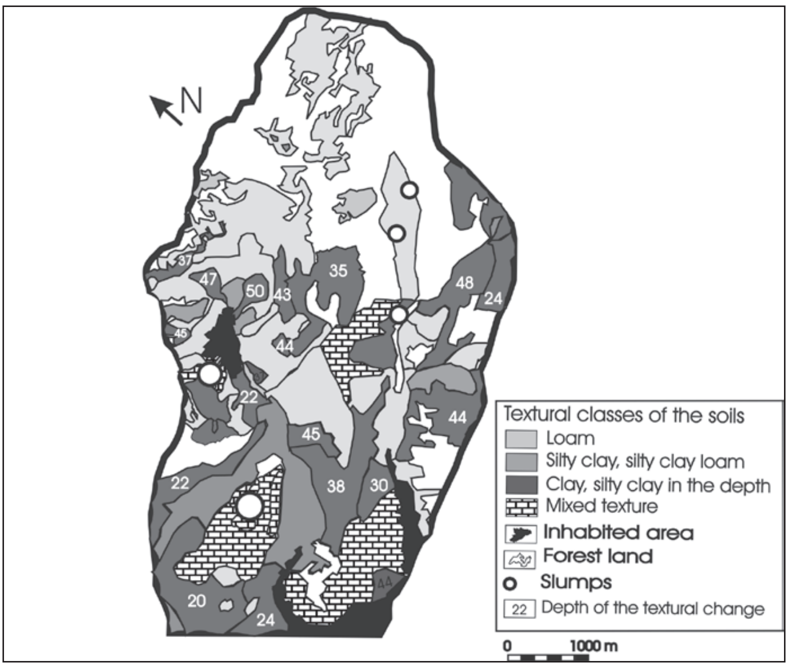

Figure 5: Textural classes of the soils in the Măgherani Basin (soils of areas covered by woods were not investigated)

Determination of grain-size distribution of the samples in the area of the slumps is sometimes rather difficult due to the mixing of soil horizons. Strong dissectivity increases surface erosion. Material of various grain sizes washed from the surface is accumulated in irregular setting at the foot of the steep positive forms. In such cases, grain-size distribution is so variable even in small areas that it cannot be plotted on large-scale maps. These areas are marked as regions with mixed-textured soils. In the course of the laboratory analysis of the soils, the followings were experienced:

- Iron oxides are present only in small quantity in the soil-forming rocks; therefore, cementing of the grains is relatively poor.

- Significant amount of calcium carbonate (5–18%) results in high pH (7.2–8.4). A significant portion of the soils in the area (around 60%) therefore shows neutral or basic pH.

There is a close correlation between the distribution of slumps and the pH of the soils. Practically, all slumps were formed in basic soils (Fig. 6).

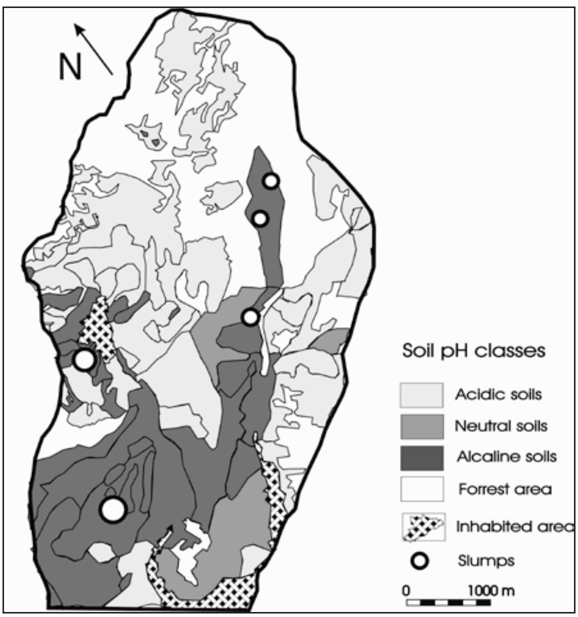

Figure 6: pH classes of the soils in the Măgherani Basin (soils of areas covered by woods were not investigated)

The significance of calcium carbonate is especially relevant in the case of the slopes with southern, southwestern and western orientation, where it is found mostly on or near the surface. These sunny slopes have strong insolation; long dry periods are frequent, especially in July and August, when the biological activity of the soils virtually ceases. In this case, the CO_2 content of the soil air is almost the same as that of the atmosphere, i.e. much smaller than in those soils characterized by a high grade of biological activity. In the course of a heavy downpour following such a dry period, the soil becomes saturated rapidly; the balance between the $CaCO_3$ content and the soil moisture breaks rapidly while the low CO_2 content remains.

Such conditions result in the increase of the pH of the soil causing the occurrence of excess negative charge along the edges of the clay particles. Therefore, they start to dissociate and push each other away (Schuylenborgh, 1972) [4].

Generally, this state remains for a short period of time, maybe only for a couple of hours. If this relatively short period coincides with the presence of a factor triggering a landslide (e.g., overload on a slope), the landslide may take place. Such coincidence can occur several times in a decade. Based on the above, it can be stated that low shear strength and low cohesion conditions, which can result in the formation of slumps, may occur due solely to the chemical processes taking place in the interior of the rocks.

References

[1] Chleborad, A. F., Baum, R. L. and Godt, J. P. (2006), Rainfall thresholds for forecasting landslides in the Seattle, Washington, Area-exceedance and probability. U.S. Geological Survey Open-File Report 2006–1064.

[2] Chung C. F., Fabbri A. G. (1999), Probabilistic prediction model for landslide hazard mapping. *Photogrammetric Engineering and Remote Sensing* 65: 1389–99.

[3] Coe, J. A., Michael, J. A., Crovelli, R. A., Savage, W. Z., Laprade, W. T., Nashem, W. D. (2004), Probabilistic assessment of precipitation-triggered landslides using historical records of landslide occurrence, Seattle, Washington. *Environmental and Engineering Geoscience* 10 (2), 103–122.

[4] Schuylenborgh, J. van (1972), Short introduction to the physico-chemical aspects of weathering and soil formation. *Lecture notes I.T.C. Enschede.*

[5] Montgomery, D. R., Sullivan, K., and Greenberg, H. M. (1998), Regional test of a model for shallow landsliding. *Hydrological Processes* 12: 943–55.

[6] Swanson, F. J. and Dryness, C. T. (1975), Impact of clear-cutting and road construction of soil erosion by landslides in the western Cascade Range, Oregon. *Geology* 3: 393–6.

[7] Ujvari, J., Buz, V. (1973), Perioade caracteristice de supraumectare a apelor subterane şi procesele gravitaţionale de versant, *Studia Univ. Babeş-Bolyai, seria Geographia*, fasc. 2, Cluj-Napoca.

[8] Varnes, D. J. (1984), Landslide Hazard Zonation: *A Review of Principles and Practice.* Paris, UNESCO Press, 63 pp.

[9] Zezere, J. L., Trigo, R. M., and Trigo, I. F. (2005), Shallow and deep landslides induced by rainfall in the Lisbon region (Portugal): assessment of relationships with the North Atlantic Oscillation, *Nat. Hazards Earth Syst. Sci.*, 5, 331–344.

Acta Universitatis Sapientiae
Agriculture and Environment, **5**, (2013) 80-90

Application of molecular markers in medicinal plant studies

Alireza POURMOHAMMAD
email: pourmohammad@ymail.com

University of Maragheh, Faculty of Agriculture,
Department of Agronomy and Plant Breeding,
Maragheh, Iran

Abstract. The World Health Organization has estimated that more than 80% of the world's population in developing countries depends primarily on herbal medicine for basic healthcare needs. Approximately two thirds of the 50 000 different medicinal plant species in use are collected from the wild and only 10% of medicinal species used commercially are cultivated. DNA-based molecular markers have utility in the fields like taxonomy, physiology, embryology, genetics, etc. DNA-based techniques have been widely used for authentication of plant species of medicinal importance. The geographical conditions affect the active constituents of the medicinal plant and hence their activity profiles. Many researchers have studied geographical variation at the genetic level. Estimates of genetic diversity are also important in designing crop improvement programmes for the management of germplasm and evolving conservation strategies. The DNA-based molecular marker helps in the improvement of medicinal plant species. DNA markers are more reliable because the genetic information is unique for each species and is independent of age, physiological conditions and environmental factors.

Keywords: polymorphism, authentication, identification, genetic variation

Introduction

With the cultivation of medicinal plants, traditional breeding techniques and biotechnology can be used to identify the genetic improvement of the growth of the plants [7]. Selections based on molecular markers have been used widely in crop improvement. This method is desirable, genotypes detected in the early stages of the selection process will be expedited. This means that certain DNA sequences or alleles identify genes that are directly associated with the trait. Identifying functional genes and associating sequences with them is a costly process, but technical progress and results of whole-genome sequencing of model plants, such as *Oryza sativa* and *Arabidopsis thaliana*, is facilitated. There is high degree of similarity between the functional genes in the DNA sequences. Thus, DNA probes from one species can be used for the sequencing of related species. Availability of complete genome sequences for rice and *Arabidopsis* and the rapid increase in genomic resources for several other models such as *Medicago*, *Lycopersicon* and *Populus* has provided new opportunity for medicinal plant breeding through comparative genomics [15, 25].

A genetic marker is a gene or sequence with specific place on a chromosome which is associated with a particular trait [38]. The efficiency of molecular markers depends on the detected polymorphism [22, 31, 32].

Purpose

The purpose of this study is the evaluation of different DNA markers in classification, identification and breeding of medicinal plants.

DNA markers in medicinal plants

RFLP[1]

Restriction fragment length of polymorphisms (RFLPs) were one of the first markers that were used to detect variation in a DNA sequence with restriction digestion by enzymes. One of the newest methods for the identification of individual diversity in nucleotides of DNA can be conceived directly through single-nucleotide polymorphism. Jana and Eva [19] performed RFLP analysis of the *Hypericum perforatum* L. They expressed that similar RFLP patterns in some somaclonal plants of R0 are related to the apomictic method of reproduction. RFLP patterns compared with parental plants showed that some offsprings have been established through sexual reproduction. Phylogenetic relationships among species of *Anthemideae* and *Asteraceae* have been studied using RAPDIS, SSR and RFLP markers in Egypt. In this study, twenty-six RFLP bands were identified after cutting with *Eco*RI and *Bam*HI, and the

[1] Restriction Fragment Length Polymorphism

similarity mean was between 0 and 0.5. Digested DNA products of *Achillea fragrantissima, Artemisia arborescens, Artemisia Judaica, Matricaria aurea, Glebionis Coronaria, Cotula barbata* and *Cotula cinerea* were 6, 4, 5, 4, 3, 2 and 2, respectively, while *Achillea santolina, Anacyclus monanthos* and *Matricaria recutita* had no band [1].

The taxonomy and physiology of India *Citrus* have been studied using PCR-RFLP with trnD-trnT and rbcL-ORF regions and sequence analysis of intragenic spacer region trnD-trnT gene by Satya Narayan et al. [36]. In this experiment, 50 samples of genotype collected plant material of wild, semi-wild and domesticated. PCR-RFLP analysis generated of phylogenetic tree with moderate to high bootstrap values while trees based on trnL-trnF sequence showed only moderate to low bootstrap, which reflects the unknown origin of some *Citrus* genotypes. Nevertheless, the PCR-RFLP and trnL-trnF data distinguished *Citrus maxima*, *C. medica* and *C. Citrus* from each other.

AFLP[2]

AFLP markers have been successfully used for linkage mapping with high saturation in *Lolium perenne* L. AFLP markers are done in *Lolium* genomic mapping as a prelude in the determination of agronomic traits QTLs and marker-assisted selection [6]. AFLP analysis is used in genus *Ocimum* species by Labra et al. [21]. *Ocimum* genus is over 150 species. They concluded that the combined analysis of morphological characteristics, composition of volatile oils and molecular markers is a robust method for the identification of classification and its correlation with agronomic traits. In this study, the genetic distances were calculated with Nei and Li index [6]. Genetic relationships among *Rosa damascena* plants were studied by AFLP markers in Turkey [5]. There was no polymorphism between plants and marker patterns derived from different plants were similar.

The collected data showed that all *R. damascene* plants derived from the same original genotype vegetative reproduction.

RAPD[3]

RAPD technique is used by Salim khan et al. for the authentication of *Glycyrrhiza glabra* L., differentiating it from *Abrus precatorius* L. [35]. Fifty-two primers were screened for identifying original and unfathered samples. Six-

[2]Amplified Fragment Length Polymorphism
[3]Rapid Amplification of Polymorphic DNA

teen primers generated reproducible and specific amplification product. The PCR amplification products were clearly distinguished original and counterfeit samples with similar morphology. So, RAPD can be used as a complementary tool in quality control [34].

In a study by Mehrnia et al. [24], fresh leaves of 20 plants belonging to Iranian *Astragalus microcephalus* were gathered and studied with RAPD markers. The results showed that RAPD markers could be used to study the systematic relationship between related species. In a similar work by Sultan et al. [39], *Podophyllum hexandrum* was collected from high-latitude regions of the north-western Himalayas. The RAPD analysis showed a high degree of genetic diversity among the 12 collected samples, which can be attributed to geographical and climatic conditions. This study demonstrated that RAPD markers are very useful tools for comparing the genetic relationship and diversity patterns between medicinal plants.

SSR[4]

Genetic characterization of *Rhodiola rosea* L. was performed using SSR markers. Genetic relationships of 30 samples of *Rhodiola rosea* were studied using 10 markers. SSR analysis produced 12 polymorphic locus with an average of 1.8 polymorphic band per primer. Genetic differentiation was significantly low which indicates a high level of gene flow and a strong influence on the genetic structure. Results showed a significant gene flow between the populations of *R. rosea* [37].

SNP[5]

Coles et al. [12] used tagged, sequenced and expressed libraries to identify SNP in *Chinopodium qiuna* willd. SNP markers identified in this study had particular value in the gene expression and regulation associated with seed, while distinguished SNPs had immediate application in genetic mapping experiments, germplasm introduction and evolutionary relationships within the genus *Chenopodium.*

[4] Simple Sequence Repeats
[5] Single-nucleotide polymorphisms

Authentication of medicinal herbs using molecular markers

Techniques based on DNA were widely used for the authentication of important medicinal plants. Molecular biology provides a set of techniques that can be useful for the authentication of medicinal plants. This fact is useful particularly in plants that are indistinguishable from other species or fake varieties in terms of morphology or phytochemistry [28].

Some researchers determined AFLP technique potential to analyse the genetic distances between varieties of *Ocimum basilicum* L. and the correlation between genetic distance, the patterns of essential oils and morphological characteristics [21]. RAPD technique is used for determining the components of the medicinal plants *Astragalus membanaceus, Ledebouriella seseloides* and *Atractylodes macrocephala.* Components in pharmaceutical formulations have been identified using single RAPD primer [2].

In the study by Sultan et al. [39], three RAPD primers successfully differentiated the *Podophyllum* species from the northwest of Himalayas. In another study, three random primers were used for the detection of genetic variation in *Astragalus* medicines sold on Taiwan markets [2]. SSCP analysis of the PCR products was performed to separate the two species of *Astragalus* [24]. RAPD analysis was used for the authentication of *Glycyrrhiza glabra* L., differentiating it from *Abrus precatorius* L. Fifty-two oligonucleotide primers screened for the detection of original from fake samples. The specified PCR products clearly distinguish the original and the fake samples having similar morphology. Thus, RAPD can be used as a complementary tool for quality control [35].

The dried samples of fruit of *Lycium barbarum* were distinguished by RAPD markers [45]. RAPD technique has been used to determine the components of Chinese drug “yu-ping-feng san”. In this study, the presence of three medicinal plants was evaluated in formulation using a single primer RAPD [10]. In another study, three random primers detected genetic variation *Astragalus* on Taiwan markets. SSCP analysis of PCR products recognized two species of *Astragalus* [9]. RAPD primers identified successfully *Atractylodes* species from Chinese formulations purchased from local markets [8].

Molecular markers in herbal drug technology

RAPD markers are useful in distinguishing different samples of *Codonopsis pilosula* [14], *Allium schoenoprasum* L. [13] and *Andrographis paniculata* [29] collected from different geographical regions. Similarly, various samples of *Arabidopsis thaliana* [4] are differentiated with ISSR markers.

Inter and intra species diversity have been studied using molecular markers. Interspecies diversity using RFLP and RAPD markers has been studied in general such as *Glycerrhiza* [44], *Echinacea* [20] and *Arabidopsis* [23]. RAPD and RFLP markers for the characterization of species *Epimedium* [26] have been used on genetic level. Members of three different species of *Scutellaria* [18] and three sub-species of *Melissa officinals* [43] were detected with RAPD. Varietal identification and genetic purity of hemp was performed using RAPD and morphological data [11].

RFLP technique was used to assess the genetic diversity intraspecies in the genus *Capsicum* and the DNA fingerprinting of pepper varieties [33]. RAPD marker was used as a tool to explore the diversity of *Simmondsia chinensis* L. Schneider [3], *Vitis vinifera* L. [40] and *Camellia sinesis* [42]. Genetic diversity within and between relationships of Withania [27] and the genetic relationships between wild relatives of *Caricaceae* was revealed using AFLP markers [41]. RAPD markers are used for genetic linkage mapping *Grandis Eucalyptus* and *Eucalyptus urophylla* [17]. RAPD markers are used for the genetic mapping of *Taxus bravifolia* Nutt. [16]. AFLP physical map of *Arabidopsis* genome is provided by AFLP marker [30].

Conclusion

Markers are different in their ability to differentiate, their mechanism of polymorphism and their genomic coverage. So, they can complement each other depending on the available techniques. Molecular technology provides an independent way to describe the medicinal plant materials.

References

[1] Abd EI-Twab MH, Zahran F. A. (2010), RAPD, ISSR and RFLP analysis of phylogenetic relationships among congeneric species (*Anthemideae, Asteraceae*) in Egpyt. International Journal of Botany 6: 1–10.

[2] Adiguzel A., Agar G., Baris O., Medine G., Fikrettin S., Meryem S. (2006), RAPD and FAME analyses of *Astragalus* species growing in eastern Anatolia region of Turkey. Biochemical Systematics and Ecology 34: 424–432.

[3] Amarger V., Mercier L. (1995), Molecular analysis of RAPD DNA based markers: Their potential use for the detection of genetic variability in jojoba (*Simmondsia chinensis* L. Schneider). Biochimie 72: 931–936.

[4] Barth S., Melchinger A. E., Lubberstedt T. (2002), Genetic diversity in *Arabidopsis thaliana* L. Heynh. investigated by cleaved amplified polymorphic sequence (CAPS) and inter-simple sequence repeat (ISSR) markers. Molecular Ecololgy 11: 495–505.

[5] Bayder N. G., Bayder H., Debener T. (2004), Analysis of genetic relationships among *Rosa damascena* plants grown in Turkey by using AFLP and microsatellite markers. Journal of Biotechnology 111: 263–269.

[6] Bert P. F., Charmet G., Sourdille P., Hayward M. D., Balfourier F. (1999), A highdensity molecular map for ryegrass (*Lolium perenne*) using AFLP markers. Theoretical and Applied Genetics 99: 445–452.

[7] Braga R. A., Dal Fabbro I. M., Borem F. M., Rabelo G., Arizaga R., Rabal H. J., Trivi M. (2003), Assessment of seed viability by laser speckle technology. Biosystems Engineering 86: 287–294.

[8] Chen K. T., Su Y. C., Lin J. G., Hsin L. H., Su Y. P., Su C. H., Li S. Y., Cheng J. H., Mao S. J. T. (2001), Identification of *Atractylodes* plants in Chinese herbs and formulations by random amplified polymorphic DNA. Acta Pharmacologica Sinica 22: 493–497.

[9] Cheng K. T., Su B., Chen C. T., Lin C. C.)2000, RAPD analysis of *Astragalus* medicines marketed in Taiwan. The American Journal of Chinese Medicine 28: 273–278.

[10] Cheng K. T., Tsay H. S., Chen C. F., Chou T. W. (1998), Determination of the components in a Chinese prescription, Yu-Ping-Feng San, by RAPD analysis. Planta Medica 64: 563–565.

[11] Cheng Z., Lu B. R., Baldwin B. S., Sameshima K., Chen J. K. (2002). Comparative studies of genetic diversity in kenaf (*Hibiscus cannabinus* L.) varieties based on analysis of agronomic and RAPD data. Hereditas 136: 231–239.

[12] Coles N. D., Coleman C. E., Christensen S. A., Jellen E. N., Stevens M. R., Bonifacio A., Rojas-Beltran J. A., Fairbanks D. J., Maughan P. J. (2005), Development and use of an expressed sequenced tag library in quinoa (*Chenopodium quinoa* Willd.) for the discovery of single nucleotide polymorphisms. Plant Science 168: 439–447.

[13] Friesen N., Blattner, F. R. (1999), RAPD analysis reveals geographic differentiations within *Allium schoenoprasum* L. (Alliaceae). Planta Meica 65: 157–160.

[14] Fu R. Z., Wang J., Zhang Y. B., Wang Z. T., But P. P., Li N., Shaw P. C. (1999), Differentiation of medicinal *Codonopsis* species from adulterants by polymerase chain reaction-restriction fragment length polymorphism. Planta Meica 65: 648–650.

[15] Gilmore S., Peakall R. (2003), Isolation of microsatellite markers in *Cannabis sativa* L. (Marijuana) in fibre crop varieties. Molecular Ecology Notes 3: 105–107.

[16] Gocmen B., Jermstad K. D., Neale D. B., Kaya Z. (1996), Development of random amplified polymorphic DNA markers for genetic mapping in Pacific yew (*Taxusbrevifolia*). Canadian Journal of Forest Research 26: 497–503.

[17] Grattapaglia D., Sederoff R. (1994), Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus urophylla* using a pseudo-testcross: mapping strategy and RAPD markers. Genetics 137: 1121–1137.

[18] Hosokawa K., Minami M., Kawahara K., Nakamura I., Shibata T. (2000), The sequences of the spacer region between the atpF and atpA genes in the plastid genome allows discrimination among three varieties of medicinal *Angelica*. Planta Medica 66: 270–272.

[19] Jana H., Eva C., (1997), RFLP analysis of *Hypericum perforatum* L. somaclones and their progenies. Euphytica 95: 229–235.

[20] Kapteyn J., Goldsbrough B., Simon E. (2002), Genetic relationships and diversity of commercially relevant *Echinacea* species. Theoretical and Applied Genetics 105: 369–376.

[21] Labra M., Mariangela M., Bernardetta L., Fabrizio G., Mauro M., Francesco S., (2004), Morphological characterization, essential oil com-

position and DNA genotyping of *Ocimum basilicum* L. cultivars. Plant Science 167: 725–731.

[22] Leela T., Suhas P. W., Seetha K., Naresh B., Thakur K. S., David A. H., Prathibha Devi, Rajeev K. V. (2009), AFLP-based molecular characterization of an elite germplasm collection of *Jatropha Curcas* L., a biofuel plant. Plant Science 176: 505–513.

[23] Lind-Hallden C., Hallden C., Sall T. (2002), Genetic variation in *Arabidopsis suecica* and its parental species *A. arenosa* and *A. thaliana*. Hereditas 136: 45–50.

[24] Mehrnia M., Shahin Z. and Ashrafoldin S. S. (2005), Intra- and inter-specific relationships within the *Astragalus microcephalus* complex (Fabaceae) using RAPD. Biochemical Systematics and Ecology. 33: 149–158.

[25] Miller Coyle H., Palmbach T., Juliano N., Ladd C., Lee H. C. (2003), An overview of DNA methods for the identification and individualization of marijuana. Croation Medical Journal 44: 315–321.

[26] Nakai R., Shoyama Y., Shiraishi S. (1996), Genetic characterization of *Epimedium* species using random amplified polymorphic DNA (RAPD) and PCR-restriction fragment length polymorphism (RFLP) diagnosis. Biological and Pharmaceutical Bulletin 19: 67–70.

[27] Negi M. S., Singh A., Laksmikumaran M. (2000), Genetic variation and relationship among and within *Withania* species as revealed by AFLP markers. Genome 43: 975–80.

[28] Ngan F., Shaw P., But P., and Wang J. (1999), Molecular authentication of *Panax* species. Phytochemistry 50: 787–791.

[29] Padmesh P., Sabu K. K., Seeni S., Pushpangadan P. (1999), The use of RAPD in assessing genetic variability in *Andrographis paniculata* Nees, a hepatoprotective drug. Current Science 76: 833–835.

[30] Peters J. L., Constandt H., Neyt P., Cnops G., Zethof J., Zabeau M., Gerats T. (2001), A physical amplified fragment-length polymorphism map of *Arabidopsis*. Plant Physiolgy 127: 1579–1589.

[31] Pourmohammad A., Moghaddam Vahed M., Khosrowchahli M., Mohammadi S. A., Yousefi A. (2010a), Genetic variation of hulless barley with agronomic traits and RAPD markers. 11^{th} Iranian crop science congress. Iran, Tehran, Environmental Science Research Institute, Shahid Beheshti University, 24–26 July 2010. (In Farsi).

[32] Pourmohammad A., Moghaddam Vahed M., Khosrowchahli M., Mohammadi S. A., Yousefi A. (2010b), Study of genetic diversity by RAPD markers and identification of informative markers for grain yield and its components in hulless barley genotypes. Seed and Plant Improvement Journal 253–267. (In Farsi).

[33] Prince J. P., Lackney V. K., Angels C., Blauth, J. R., Kyle M. M. (1995), A survey of DNA polymorphism within the genus *Capsicum* and the fingerprinting of pepper cultivars. Genome 38: 224–231.

[34] Salim Khan, Khanda J. M., Malik Z. A. (2010), Development of RAPD markers for authentication of medicinal plant *Cuscuta reflexa*. Eurasian Journal of Biosciences 4: 1–7.

[35] SalimKhan, Khanda J. M., Md Tayaab, Malik Z. A. (2009), RAPD profile for authentication of Medicinal plant *Glycyrrhiza glabra* L. Internet Journal of Food safety 11: 24–28.

[36] Satya Narayan J., Susheel Kumar and Narayanan K. Nair (2009), Molecular phylogeny in Indian *Citrus* L. (Rutaceae) inferred through PCR-RFLP and trnL-trnF sequence data of chloroplast DNA. Scientia Horticulturae. 119(4): 403–416.

[37] Soni K., Shruti R., Ankit G., Karma Y., Saurabh P., Pradeep K. N. and Harvinder Singh (2010), Genetic characterisation of *Rhodiola rosea* using gene specific SSR and CAPS molecular markers. Genetic Engineering & Biotechnology Journal 11: 1–10.

[38] Srivatsava S., Nidhi M. (2009), Genetic Markers - A cutting edge technology in herbal Drug research. Journal of Chemical and Pharmaceutical Research 1: 1–18.

[39] Sultan P., Shawl A. S., Rehman S., Fayaz Ahmed S., Ramteke P. W. (2010), Molecular characterization and marker based chemotaxonomic studies of *Podophyllum hexandrum* Royle. Fitoterapia 81: 243–247.

[40] Tessier C., David J., This P., Boursiquot J. M., Charrier A. (1999), Optimization of the choice of molecular markers for varietal identification in *Vitis vinifera* L. Theoretical and Applied Genetics 98: 171–177.

[41] Van D. B., Breyne P., Goetghebeur P., Romijn-Peeters E., Kyndt T., Gheysen G. (2002), AFLP analysis of genetic relationships among papaya and its wild relatives (*Caricaceae*) from Ecuador. Theoretical and Applied Genetics 105: 289–297.

[42] Wachira F. N., Waugh R., Hackett C. A. Powell W. (1995), Detection of genetic diversity in tea (*Camellia sinensis*) using RAPD markers. Genome 38: 201–210.

[43] Wolf H. T., Berg T. V. D., Czygan F. C., Mosandl A., Winckler T., Zundorf I., Dingermann T. (1999), Identification of *Melissa officinalis* subspecies by DNA fingerprinting. Planta Medica 65: 83–85.

[44] Yamazaki M., Sato A., Shimomura K., Saito K., Murakoshi I. (1994), Genetic relationships among *Glycyrrhiza* plants determined by RAPD AND RFLP analyses. Biological and Pharmaceutical Bulletin 17: 1529–1531.

[45] Zhang K. Y., Leung H. W., Yeung H. W., Wong R. N. (2001), Differentiation of *Lycium barbarum* from its related *Lycium* species using random amplified polymorphic DNA. Planta Medica 67: 379–381.

Acta Universitatis Sapientiae

The scientific journal of Sapientia University publishes original papers and surveys in several areas of sciences written in English.
Information about each series can be found at
http://www.acta.sapientia.ro.

Acta Universitatis Sapientiae
Agriculture and Environment

Sapientia University

Scientia Publishing House

ISSN 2065-748X
http://www.acta.sapientia.ro

Information for authors

Acta Universitatis Sapientiae, Agriculture and Environment publishes only original papers and surveys in various fields of Agriculture, especially plant breeding and protection, respectively Environment, including management, protection and evaluation. All papers are peer-reviewed.

Papers published in current and previous volumes can be found in Portable Document Format (PDF) form at the address: http://www.acta.sapientia.ro.

The submitted papers must not be considered to be published by other journals. The corresponding author is responsible to obtain the permission for publication of co-authors and of the authorities of institutes, if needed. The Editorial Board is disclaiming any responsibility.

The paper should be submitted both in Word.DOC and PDF format. The submitted PDF document will be used as a reference. The camera-ready journal will be prepared in PDF format by the editors. An accurate formatting is required in order to reduce subsequent changes of aspect to a minimum. The paper should be prepared on A4 paper (210×297 mm) and it must contain an abstract not exceeding 100 words.

Only papers written in English will be published in the journal (please use English spell-check). The paper should be prepared in single-column format not exceeding 12 pages in overall length, including figures, tables and references.

The manuscript must have the following subheadings:
Abstract, Keywords, Introduction, Material and Methods, Results and discussions, Conclusions, Acknowledgements, References.
The template file from http://www.acta.sapientia.ro/acta-agrenv/agrenv-main.htm may be used for details.

Submission must be made only by e-mail (acta-agrenv@acta.sapientia.ro).

One issue is offered to each author free of charge. No reprints are available.

Contact address and subscription:
Acta Universitatis Sapientiae, Agriculture and Environment
RO 400112 Cluj-Napoca, Romania
Str. Matei Corvin nr. 4.
E-mail: acta-agrenv@acta.sapientia.ro

Printed by Gloria Printing House
Director: Péter Nagy