# DIVERSITY OF SOME SORGHUM GENOTYPES EVALUATED BY AGRO-BIO-MORPHOLOGICAL TRAITS

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## ABSTRACT

The sorghum germplasm stored in Gene Bank has more than 20 genotypes of known or unknown origin and the Sorghum species are a modest valuable group of species used especially for animal production purposes. However, little is known about the extent and nature of the variability of these species. Eight genotypes local forms or cultivars, evaluating 13 quantitative agro-bio-morphological traits, were used for the assessment of genetic diversity of Sorghum genotypes. The study was carried out at the experimental field of Agricultural University of Tirana, Albania and aimed at evaluating the major agro-bio-morphological traits, characterizing the sorghum genotypes, and defining patterns of potential Sorghum forage species using PCA, correlation and cluster methods. Principal Components Analysis (PCA) and cluster analysis identified the variances of the first two principal components (78.9%) and the proportion of the total variance each factor accounts for and range Sorghum genotypes into three different cluster groups. Study analysis identifying quantitative morphological characters with most agronomic interest which account for genetic diversity will facilitate the maintenance and agronomic evaluation of the Sorghum germplasm.

***Keywords***: Sorghum genotypes, principal components, cluster analysis

## 1. INTRODUCTION

Sorghum (*Sorghum ssp.* (L.) Moench.) belongs to the Poaceae family and it is one of the most important cereal crops in the world. There are about 30 *Sorghum* species; *S. bicolor* is cultivated for grain and forage, while *S. halepense* (L.) Pers. (Johnson grass) and *S. propinquum* (Kunth) Hitchc., are cultivated only for forage. Most species are annuals although some are perennial. Sorghum stems may reach over 4 m height, the inflorescence, with small grains of 3–4 mm diameter, varies greatly in size and shape (IBPGR and ICRISAT 1993).

Sorghum is extensively grown in arid and semi-arid tropical and subtropical regions of the world (Doggett, 1988). FAO (2012) stated that the Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important cereal crop in the world. Sorghum crop ranks fifth in production after wheat, maize, rice, and barley and has a predominant contribution towards food and fodder security in the arid and semi-arid regions of the world (Chittapur *et. al*., 2015).

Sorghum, considered as an important part of the diet for many of the world's population, is also used as a forage crop (Bioversity 2010) and for industrial purpose (sweet sorghum) to produce sorghum syrup. Cultivated sorghum [*Sorghum bicolor* (L.) Moench] is an annual C4 photosynthetic monocotyledonous plant (Mutegi *et. al*., 2010), and it will remain a basic staple food for many rural communities.

Sorghum, widely cultivated especially on shallow and heavy clay soils, is extremely drought-tolerant, making it an excellent choice for semi-arid and dry areas. In recent years, there has been a shift in sorghum production from the drier production areas to the wetter areas. This change has resulted in the identification and development of genotypes which are more tolerant to lower temperatures (Bernardo 2010; Alina *et. al*., 2017).

Sorghum yield increase (grain and forage) is of primary interest in food security for an increasing world population, and especially for millions of rural families in the arid and semi-arid areas of the world. Yield depends largely on the ability of plant breeders to increase sorghum grain yield and forage, which depends on several yield components (Gerrano *et al.*, 2014).

Sorghum yield improvement involves the identification and dissection of the most important agronomic yield components (Gixhari, *et. al*., 2014). Akatwijuka *et. al*., (2016) and Ramya *et. al*., (2017) reported that sorghum grain yield has low heritability, so, improvement of yield remains one of the major breeding objectives of many cereal improvement programs. Traditionally, germplasm diversity is evaluated by morphological descriptors (Gixhari *et. al*., 2013; Gixhari *et. al*., 2014), which remain the only legitimate marker types accepted by the International Union for the Protection of New Varieties of Plants (UPOV 2012).

The Albanian germplasm of the sorghum collection is a modest valuable group of forage species for animal production. Despite its economic importance, however, sorghum has not been characterized very well genetically, and little is known about the extent and nature of the variability of these species. Therefore, the information about extent of uses of various gene pools are extremely valuable for the rational planning of the use of germplasm in breeding programs (Gixhari *et. al*., 2016).

The present study analyses the genetic diversity among sorghum genotypes and investigates the association among the most important morphological characters, to aid in the selection and more efficient use of sorghum germplasm in breeding programs.

## 2. MATERIALS AND METHODS

*Plant material and cultural practices:* Eight sorghum genotypes: AGB2642, AGB2647, AGB2650, AGB3077, AGB3078, AGB3097, and two collected genotypes AGB-1 and AGB-2 were investigated.

*Cultural practices:* Sowing date and growing conditions as the distance between plants in a row and between rows, fertilizer application, number of plants established, plant protection, harvest date etc. were the same for each genotype and consistent with established farming practices of the area and with the variety used.

*Agro-morphological characters*: Plant height (PH) (cm), Number of internodes (NI), number of leafs (NL), leaf length (LL), leaf width (LW) (cm), panicle (inflorescence) length (PL) (cm), number of plants (NP), field germination (FG) (percentage), days to germination (DG), days to flowering (DF), panicle flowering duration days (PFD), days to maturity (DM), days to harvesting (DH).

*The experimental site and field observations*: The study for the assessment of sorghum diversity was carried out at the experimental field of Agriculture University of Tirana (latitude: 402405N; longitude: 0194108E; elevation: 40 m) during two growing seasons (2017, 2018).

*Field observations*: The descriptors used for evaluation of sorghum species include quantitative and qualitative (or coded) plant characters or descriptors) (IBPGR and ICRISAT. 1993). Field observations and evaluation of qualitative traits were realized on 25 plants of each plot.

*Statistical Analysis*: The differences between sorghum genotypes for the mean values of the bio-morphological quantitative characters observed and evaluated were carried out using ANOVA analysis. Identification of sorghum genotypes of relatively similar characteristics or genetic distances and identification of the most important agro bio-morphological characters that influence highly on the total variation, was realized using Principal Components Analysis (PCA) on correlation and cluster analysis methods. All statistics data for bio-morphological characters were calculated employing the SAS JMP Statistical Discovery (2012), and a dendrogram (ward method) and two-dimensional relationship diagram (sorghum genotypes x morphological characters) were carried out.

## 3. RESULTS AND DISCUSSIONS

*Analysis of morphological quantitative characters*: ANOVA analysis found the presence of significant differences between sorghum genotypes for the most of bio-morphological characters at the probability $P_{0.05}$. High degree of variation was observed for DM, DH, PF, DF, LW, DG, NP and FG characters. Moderate differences were found for LL and PH characters, and no significant differences, at the probability $P_{0.05}$, for NL, NI and PL characters.

***Principal Components Analysis***: Principal Components Analysis on Correlations identified the variances of the principal components and the proportion of the total variance each factor accounts for. Eigenvalues and percent of variances each factor accounts for are in table 1 reported. Based on the mineigen criterion (Kaiser 1960) three principal components that account for 93.38% of the total variation are retained for further analysis. PCA results show that the major sources of variation in the measurements are given by the first two PCs. All quantitative variables contribute to 100% of total variation.

The percentages of total variation accounted for by each of the first three PCs are 49.2%; 29.7% and 14.4% respectively (Table 1). The first three PCs explain 93.38% of the original variation, and the variation > 80.0% is acceptable for characterization and evaluation of plant collections in a Gene Bank (Jolliffe 2002).

**Table 1.** Eigenvalues matrix of principal components (8 sorghum genotypes x 13 agro-morphological characters)

| Principal Components/factor analysis | | | | | | |
|---|---|---|---|---|---|---|
| PC No. | Eigenvalue | Percent variance | Cumulative Percent | $\chi^2$ | df | Prob. > $\chi^2$ |
| 1 | 6.4025 | **49.2** | **49.2** | 319.781 | 76.698 | <.0001* |
| 2 | 3.8647 | **29.7** | **78.9** | 233.008 | 75.169 | <.0001* |
| 3 | 1.8723 | 14.4 | **93.381** | 151.131 | 69.270 | <.0001* |
| 4 | 0.3998 | 3.076 | 96.457 | 76.809 | 60.179 | 0.0729 |

$\chi^2$ – Chi Square, df – degree of freedom; Prob. – probability; *significance level equal to the 0.05 of probability

The maximum information from agro-morphological data was received using ordination methods in combination with cluster analyses (Jolliffe 2002). Dimensional scaling of relationships (sorghum genotypes x bio-morphological characters) that accounts for the larger proportion of the total variance in PC1, PC2 and PC3 revealed by PCA indicate that the contribution of each sorghum genotypes and of each quantitative agro-morphological character on the total of variation was found inequal.

There were five sorghum genotypes included in PC1 and PC2 (AGB2647, AGB2650, AGB3097, AGB-1 and AGB-2) that account for 78.9% of total variation, and three sorghum genotypes in PC3 (AGB2642, AGB3077 and AGB3078) which contribute with only 14.4% on the total variation (Table 1; Figure 1).

For PC1 (with 49.2% contribute on the total variation) characters as DM, DH, PF, DF, LW and DG were the most important source for the variation of PC1 (Figure 1). Four agro-morphological characters (PF, DF, LW and DG) with nearly the same value of eigenvectors are the same important to the PC1 (Table 2, Figure 1). The characters as FG, NP and PH showed important negative influence on the PC1 variance (Figure 1).

Variation in Component two (PC2 = 29.7% of total variation) was mainly result of differences in LL, NI and NL bio-morphological characters. Characters as DG, DF, DH and PH account for nearly the same amount of variance on PC2. The characters PL showed important negative influence on the PC2 variance (Table 2, Figure 1).

For PC3 (with only 14.4% contribute on the total variation) characters as DH, NL and NI were the most important source for the variation on the PC3 variance. The characters as PF and PH showed important negative influence on the PC3 variance (Table 2, Figure 1).

**Table 2.** Matrix of vectors of three PC for 8 sorghum genotypes x 13 agro-morphological characters

| No | Agro bio-morphological characters | | PC1 | PC2 | PC3 |
|---|---|---|---|---|---|
| 1 | Plant height | PH | -0.27410 | 0.28535 | 0.27098 |
| 2 | Number of internodes | NI | -0.16944 | **0.34332** | **0.39076** |
| 3 | Number of leafs | NL | -0.17050 | **0.34154** | **0.39322** |
| 4 | Leaf length | LL | -0.23570 | **0.35050** | 0.11193 |
| 5 | Leaf width | LW | **0.31499** | -0.19995 | 0.24819 |
| 6 | Panicle length | PL | 0.05646 | -0.28717 | **0.53684** |
| 7 | Number of plants | NP | **-0.30998** | 0.14928 | **-0.34085** |
| 8 | Field germination | FG | **-0.31023** | 0.14923 | **-0.34023** |
| 9 | Days to germination | DG | **0.31106** | 0.29887 | -0.13057 |
| 10 | Days to flowering | DF | **0.31866** | 0.29410 | -0.06771 |
| 11 | Panicle flowering duration | PF | **0.31909** | 0.28446 | -0.01294 |
| 12 | Days to maturity | DM | **0.33368** | 0.23586 | -0.00349 |
| 13 | Days to harvesting | DH | **0.32454** | 0.28607 | -0.05638 |

In bold all eigenvectors > 0.30

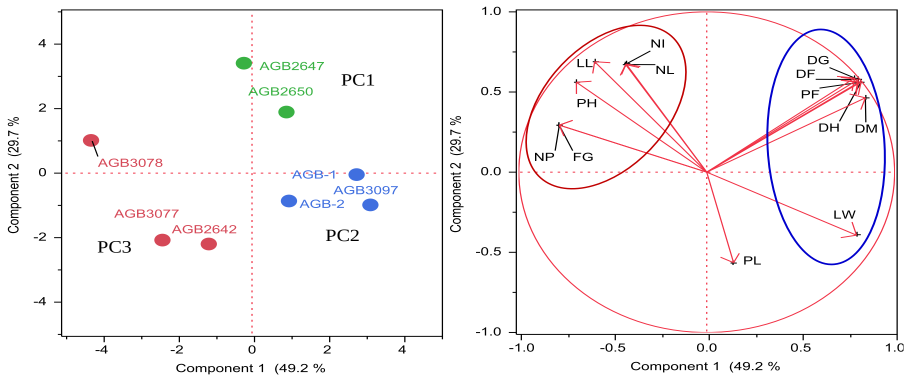



**Fig. 1:** Relationships among 8 sorghum genotypes based on agro-morphological characters revealed by PCA.

***Cluster analysis***: Relationships between 8 sorghum genotypes assessed by agro-morphological characters and genetic similarity/distances coefficients revealed by cluster analyses categorized all sorghum genotypes into three clusters (Figure 2).

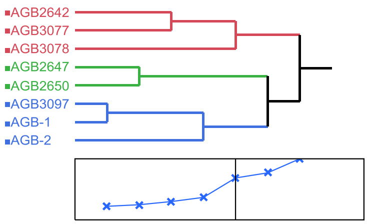
All three clusters were differentiated especially by eight bio-morphological characters of PC1, and three characters of PC2.

The first cluster consists of two sorghum genotypes (AGB2647, AGB2650) and was differentiated by DM, DH, PF, DF, LW and DG agro-bio-morphological characters of PC1. Correlation analysis found strong positive correlation between these characters (r ranges from 0.70 to 0.94). Elangovan *et. al*., (2013) and Verma *et. al*., (2017) reported similar results for the most of these traits.

Only LW character showed negative correlation with all other characters (r ranges from -0.41 to -0.72).

The second cluster includes three sorghum genotypes (AGB3097, AGB-1 and AGB-2) and were differentiated by LL, NI and NL quantitative morphological characters of PC2. These three characters showed strong positive correlation between them (r range from 0.71 to 0.98). Durrishahwar *et. al*., (2012) found low and negative correlation among these traits.

The third cluster includes three sorghum genotypes (AGB2642, AGB3077 and AGB3078) and were differentiated by DH, NL and NI bio-morphological characters of PC3 (Figure 2).




| Clustering History | | | |
|---|---|---|---|
| **Clusters** | **Distance** | **Leader** | **Joiner** |
| 7 | 1.367070341 | AGB3097 | AGB-1 |
| 6 | 1.525840283 | AGB2647 | AGB2650 |
| 5 | 1.846136138 | AGB2642 | AGB3077 |
| 4 | 2.265843250 | AGB3097 | AGB-2 |
| 3 | 4.226051924 | AGB2642 | AGB3078 |
| 2 | 4.741174084 | AGB2647 | AGB3097 |
| 1 | 6.158118161 | AGB2642 | AGB2647 |

**Fig. 2:** Dendrogram of relationships among sorghum genotypes and bio-morphological characters.

Highest distance was found between AGB2642 (leader) and AGB2647 (joiner) sorghum genotypes, and low distance (higher similarity) was found among sorghum genotypes as AGB3097 (leader) and AGB-1 (joiner) (Figure 2).

The data about genetic similarity and distances provides a better information on germplasm sampling. Separation of sorghum genotypes into three clusters helps select the parents for crosses and gene introgression from distantly related germplasm. The presence of some interrelationships among quantitative morphological characters of different sorghum genotypes suggests the biological status origin of these sorghum genotypes is their breeding pedigree (Gixhari *et. al.*, 2014) or research materials status. No one of these sorghum genotypes fulfil the characteristics of an advanced or improved cultivar.

Study identified the agro bio-morphological characters with more significant weighting on respective PC1 and PC2 variances (DM, DH, PF, DF, LW, DG, LL, NI and NL characters), which can be used successfully as morphological quantitative markers for evaluation and characterization of the sorghum germplasm.

The amount of genetic variability found in the present study, available to the breeders, is sufficiently helpful for the selection of desirable characters. This variability serves as a possible reserve of desirable traits (genes), and it is a valuable source for creation of new favourable gene combinations to sustain field sorghum breeding programs.

## 4. CONCLUSION

The field evaluation test in the present study addresses the first characterization of sorghum genotypes and the identification of the most important agro-morphological diversity and determination of the patterns of sorghum genotypes with high forage value.

PCA results showed that the first three PCs account for a substantial proportion of total variation, 93.4%. The percentages of total variation accounted for by each of the first three PCs were 49.2%, 29.7% and 14.4%, respectively.

PCA and cluster analysis identified the agro bio-morphological characters with more significant weighting on respective PC1 and PC2 variances (DM, DH, PF, DF, LW, DG, LL, NI and NL characters), which can be used successfully as morphological quantitative markers for evaluation and characterization of the sorghum germplasm.

The amount of genetic variability found in the present study helps sufficiently select the desirable characters and serves as a valuable source for creation of new gene combinations to sustain field sorghum breeding programs.

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# GENETIC VARIABILITY BETWEEN SOME ALBANIAN NATURAL POPULATIONS AND COMMERCIAL CULTIVARS OF *SALVIA OFFICINALIS* L. BASED ON RAPDs

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## ABSTRACT

Dalmatian sage (*Salvia officinalis* L.) represents one of the most significant aromatic plants that naturally grow in Albania and most of Mediterranean region. Nowadays, due to the essential oils and because of economical, medicinal, aromatic and culinary importance, it is cultivated in some areas of the country. Different molecular markers have been successfully used for years to evaluate genetic variability of common sage. The present study evaluates the genetic relationships between six natural populations and six commercial cultivars of *Salvia officinalis* in Albania. Genomic DNA was isolated based on a modified CTAB protocol and PCR amplification was completed, using ten decameric random primers from Operon Technologies. The PcoA software was used to perform cluster analysis and determine genetic distances among the populations. Results showed a clear separation between the indigenous and cultivated groups, and higher genetic diversity is found within natural populations. These data show that native common sage plants represent a genetic pool different from that of commercial ones sharing a similarity of only 25%.
***Keywords:*** sage, molecular markers, genetic variability, RAPD markers

## 1. INTRODUCTION

*Salvia officinalis* L. is an outcrossing, insect-pollinated, long-lived sub-shrubby plant of the family *Lamiaceae*, and it has been known for its medicinal and culinary uses since ancient times. Common sageis native to the east side of Adriatic (Ristić *et. al*., 1999) and Ionian seas with a habitat reaching south into northwest Greece (Karousou *et. al*., 2000).

These species have been investigated mainly for the content and composition of the essential oil, since this has proved to be responsible for most of the pharmacological effects attributed to the plant (Jug-Dujaković *et. al*., 2012; Liber *et al*., 2014). Sage is valued because of its antiseptic, antiinflammatory, antioxidant, carminative, cholagogue and diaphoretic properties (Pluhár *et. al*., 2012).

Its autochthonous region is the Mediterranean basin, along with a large number of endemic species that make it one of the world plant biodiversity centers (Medail and Quezel, 1999; Sales *et al*., 2001). It has been harvested mostly from wild populations in Croatia, Bosnia and Herzegovina, Montenegro and Albania.

Meanwhile, Sage (*Salvia officinalis*) naturally grown in Albania, is studied and used extensively for commercial purposes. It is also cultivated in a number of private companies for the high quality of the essential oils. Considering the appropriate conditions, the market demand and the economic interest of cultivation, sage is successfully cultivated in a considerable area of around 1500 ha, giving these areas a considerable opportunity for economic and social development.

Molecular markers provide a powerful tool for proper characterization of plant germplasm and their management. Among developed genetic markers, RAPDs, AFLPs and SSR, RFLP, have been widely used for genetic diversity analyses (Bacu *et. al*., 2005; Khalil 2005; Adhikari 2013; Papa *et. al.*, 2016) among sage populations of Albania.

Previous studies of molecular characterization with RAPD markers, AFLP and SSR markers for 80 genotypes of Albanian sage populations (Bacu *et al*., 2005; Bacu *et al*., 2011) proved that genotypes of the same populations shared from 30% to 60% and to 80% similarity; that the populations of the near geographical locations grouped together giving this way a strong indication on the important role of the environmental conditions into the genome of this species.

In other studies (Papa *et al*., 2016; Papa *et al*., 2017), 43 genotypes for 13 natural populations of northern Albania were evaluated for the diversity of monoterpene synthases coding genes and PCR-RFLP of cp-DNA. Results from both methods showed that genotypes of the same populations shared 65% to 80% similarity, leaving aside as the most distinctive one the populations of Kruja, Torovica and Prostriba (Papa *et al*., 2016; Papa *et al*., 2017).

However, sage has been cultivated intensively in North and South of the country for export purposes dedicated to their essential oils. This commercialization has reduced the appraisal of vegetation only in the GC definition of the essential oils content, which accompanies this product as a companion certificate to foreign markets (Kathe et al., 2003; Kongjika et al., 2005). Thus, the genetic variation between natural populations and cultivated ones with molecular markers are less exploited.

Rolim *et al*., (2011) said that random Amplified Polymorphic DNA (RAPD) technology is a fast procedure for studying genetic diversity using polymerase chain reaction. The poor reproducibility in early RAPD analysis has been largely overcome through improved laboratory techniques and band scoring procedures (Khalil *et al*., 2012). Since RAPD markers are dominant, they can be used to identify genetic variation (Solyman *et. al*., 2014), genetic diversity analysis (Maric *et al*., 2004; Papa *et al*., 2016) and phylogenetic relationships (Goriunova *et al*., 2004; Papa *et al*., 2016) by taking into account the fact that profiles are scored for the presence or absence of a single allele.

This study considers six natural and six cultivated populations of northern and southern Albania, which generally exhibit a low morphological diversity, aiming to exploit their genetic diversity.

## 2. MATERIALS AND METHODS

**Plant material:** Fresh young leaves from 12 populations of *Salvia officinalis* of northern and southern Albania (six natural and six cultivated) were used to extract total genomic DNA (Table 1). The cultivated plant material was taken from the 'ATC Natural' company, which deals with the cultivation of medicinal plants in different cities of Albania and their export to Europe.

**Table 1.** Sage germplasm used in this study, their source and origin

| No. | Populations | Latitude | Longitude | GPS coordinates |
|---|---|---|---|---|
| 1 | Shkodër | 42.066447 | 19.428860 | 42° 3' N, 19° 25' E |
| 2 | Skrapar | 40.5 | 20.216667 | 40° 30′ 0″ N, 20° 13′ 0″ E |
| 3 | Leskovik | 40.15 | 20.6 | 40° 9′ 0″ N, 20° 36′ 0″ E |
| 4 | Lezhë | 41.777724 | 19.658028 | 41° 46' N, 19° 39'E |
| 5 | Himarë | 40.101667 | 19.744722 | 40° 6′ 6″ N, 19° 44′ 41″ E |
| 6 | Koplik | 42.213611 | 19.436389 | 2° 12′ 49″ N, 19° 26′ 11″ E |

**Isolation of genomic DNA**: Equal amounts (0.1 g) of leaf tissue were placed in a mortar chilled with liquid nitrogen and were ground to fine powder. Total genomic DNA was extracted as described in (Doyle and Doyle 1987). Quality and quantity of DNA was measured based on (Sambrook *et al*., 2000).

**PCR amplification**: Ten arbitrary RAPD primers chosen from different references as described in Table 2 were used: OPA20, OPA09, OPA11, OPA08, OPA19, OPB8, OPB12, OPC15, OPB17 and OPB13.

The PCR amplifications were carried out in Veriti 96-Well Thermal Cycles (Applied Biosystem) in a total volume of 25 µl containing Master mix (Cinnagen, Tehran, Iran), genomic DNA (30 ng), 50 pmoles of decameric primer and Taq DNA polymerase. The PCR program started with an initial phase of 1 minute at 95$^o$C, followed by 45 cycles of 30 s at 95$^o$C, 30 s at 39$^o$C, 2 min at 72$^o$C and 10 min final elongation at 72$^o$C (Williams *et al*., 1990).

**Table 2.** RAPD primers sequences based on (Scoula *et al*., 1999)

| Primer | Primer Sequence |
|---|---|
| OPA20 | 5' -GTTGCGATCC- 3' |
| OPA09 | 5' -GGGTAACGCC- 3' |
| OPA11 | 5' -CAATCGCCGT- 3' |
| OPA8 | 5' -GTGACGTAGG- 3' |
| OPA19 | 5' -CAAACGTCGG- 3' |
| OPB8 | 5' -GTCCACACGG- 3' |
| OPB12 | 5' -CCTTGACGCA- 3' |
| OPC15 | 5' -GACGGATCAG- 3' |
| OPB17 | 5' -AGGGAACGAG- 3' |
| OPB13 | 5' -TTCCCCCGCT- 3' |

The amplified products were separated by electrophoresis in horizontal 1.5% agarose gels in 10XTBE. Fragments were scored as a binary variable, (1) for the presence, and (0) for the absence of each band. The most distinct well-resolved stable bands were considered for statistical analysis.The variables were used to build genetic dendrograms with UPGMA method of PcoA software.

## 3. RESULTS AND DISCUSSIONS

The RAPDs based analysis of six natural populations and six cultivated populations of sage of Albania displayed a variable level of similarity for different primers used to amplify arbitrary primed regions. Six decameric primers of Operon technologies out of ten gave best stable polymorphic profiles, respectively OPA11, OPA08, OPA09, OPB12, OPC15 and OPB08, which were further processed (Fig 1-4). The other 4 primers, respectively OPA20, OPA19, OPB17, OPB13, did not amplify or present good amplification pattern. Table 3 shows the sequences of RAPD primers with the best polymorphic profiles, their sequence, total number of bands and polymorphic bands as well as the range of the size of fragments.

The number of amplification products per primer varied from 10 (OPB-08 and OPC15) to 12 (OPA-11). Six out of ten primers used were able to distinguish all the populations, and produced a result according to which 88% of the bands were polymorphic. This percentage of polymorphism was within the range previously reported for *Salvia fruticosa* (Skoula *et al.*, 1999), and other medicinal and aromatic species of the *Lamiaceae* family such as *Ocimum gratissimum* (Vieira *et al.*, 2001), *Lavandula angustifolia* (Echeverrigaray and Agostini 2000).

**Table 3.** List of decamer oligonucleotide primers with best polymorphic profiles

| Primer | Primer sequence 5' - 3' | Total number of bands | Nr of polymorphic bands | Size of fragments (bp) |
|---|---|---|---|---|
| OPA11 | 5' -CAATCGCCGT- 3' | 12 | 11 | 250-1200 |
| OPA08 | 5' -GTGACGTAGG- 3' | 11 | 10 | 300-1500 |
| OPA09 | 5' -GGGTAACGCC- 3' | 9 | 9 | 200-1200 |
| OPB12 | 5' -CCTTGACGCA- 3' | 11 | 9 | 250-1400 |
| OPC15 | 5' -GACGGATCAG- 3' | 10 | 8 | 150-850 |
| OPB08 | 5' -GTCCACACGG- 3' | 10 | 9 | 150-1200 |
| Total | - | 63 | 56 | - |

Figure 1-4 show the best amplified bands from six RAPD primers involved in the present investigation (Table 3).

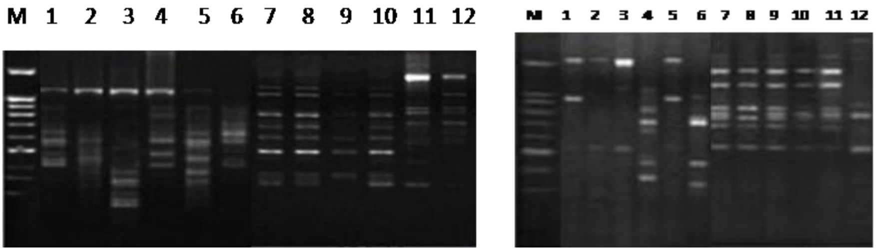


**Fig. 1:** RAPD profiles from 12 populations of sage from amplification with primer OPA11 (left) and OPA08 (right). From left to right populations: (M) marker 100bp, **1**- Himarë (natural population), **2**- Koplik (natural population) **3**- Leskovik (natural population), **4**- Lezhë (natural population), **5**- Shkodër (natural population), **6**- Skrapar (natural population), **7**- Himarë. (commercial cultivar), **8**- Koplik (commercial cultivar), **9**- Leskovik (commercial cultivar), **10**- Lezhë (commercial cultivar), **11**- Shkodër (commercial cultivar), **12**- Skrapar (commercial cultivar).

OPA11 generated 12 fragments in total, 11 of them as polymorphic. Most of the cultivated samples, based on the bands of gel electrophoresis, have similarities between them, which is expressed by the presence of bands at the same size, approximately 500-100 bp. Cultivated populations of Leskovik and Lezha (respectively 9 and 11) appear as more polymorphic among others. The size of bands generated by OPA 11 ranged from 250bp to 1200 bp. The percentage of polymorphism generated for all primer was 91%.

OPA08 generated 11 fragments totally, all of them as polymorphic. Natural populations are more polymorphic that the cultivated ones. The size of bands generated in the twelve natural populations ranged from 300bp to 1500bp. A similarity between the cultivated populations (respectively 7, 8, 9, 11 and 12) was observed, which were almost identical with each other, based on the size of bands.

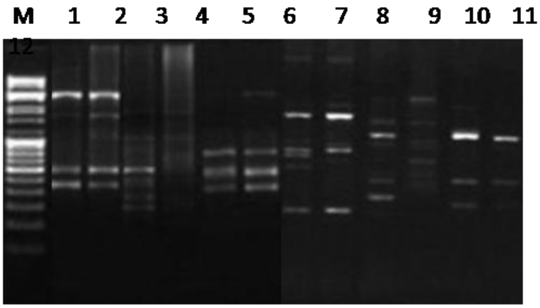


**Fig. 2:** RAPD profiles from 12 populations of sage from amplification with primer OPA09. From left to right populations: (M) marker 100bp, **1-** Himarë (natural population), **2-** Koplik (natural population) **3-** Leskovik (natural population), **4-** Lezhë (natural population), **5-** Shkodër (natural population), **6-** Skrapar (natural population), **7-** Himarë (commercial cultivar), **8-** Koplik (commercial cultivar), **9-** Leskovik (commercial cultivar), **10-** Lezhë (commercial cultivar), **11-** Shkodër (commercial cultivar), **12-** Skrapar (commercial cultivar).

OPA09 generated in total 9 fragments, all of them polymorphic. Natural populations showed a higher polymorphic level compared to the commercial cultivars. The size of bands generated ranged from 200bp to 1200bp.

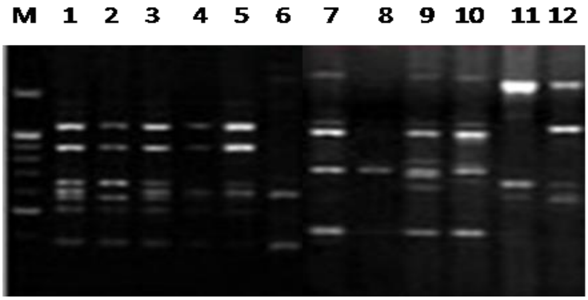


**Fig.3:** RAPD profiles from 12 populations of sage from amplification with primer OPB12: From left to right populations: (M) marker 100bp, **1-**Himarë (commercial cultivar), **2-**Koplik (commercial cultivar) **3-** Leskovik (commercial cultivar), **4-** Lezhë (commercial cultivar), **5-** Shkodër (commercial cultivar), **6-** Skrapar (commercial cultivar), **7-** Himarë (natural population), **8-** Koplik (natural population), **9-** Leskovik (natural population), **10-** Lezhë (natural population), **11-** Shkodër (natural population), **12-** Skrapar (natural population).

OPB12 generated in total 11 fragments, all of them polymorphic. A similarity between the commercial cultivars of Himara, Koplik, Leskovik and Shkodra (respectively 1, 2, 3, and 5) was observed, which were almost identical with each other. Likewise, the cultivated populations of Himara, Leskovik and Lezha (respectively 7, 9 and 10) showed a high similarity between each other. The size of bands generated from OPB12 ranged from 250 to 1400bp. The percentage of polymorphism generated for primers OPA08, OPA09 and OPB12 was 100%.

OPC15 generated in total 10 fragments, eight of them polymorphic. The size of bands generated in the twelve populations ranged from 150bp to 850bp. The percentage of polymorphism generated for primer OPC15 was the lowest compared to the others, 80 %.

OPB08 generated in total 10 fragments, 9 of them polymorphic. The natural population of Himara and the cultivar of Lezha showed higher polymorphic level compared to the rest of natural and cultivated populations. The size of bands generated from OPB08 ranged from 100bp to 1200bp and the percentage of polymorphism generated for primer OPC15 was approximately 90%.

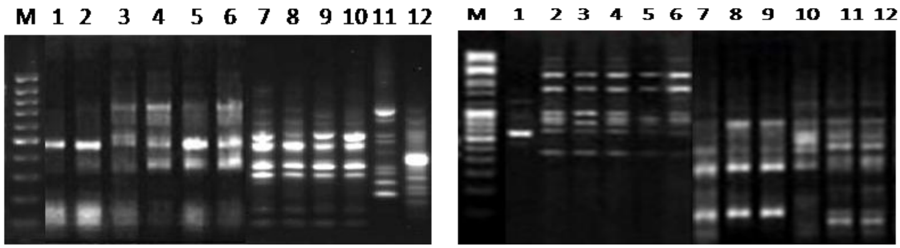


**Fig.4:** RAPD profiles from 12 populations of sage from amplification with primer OPC15(left) and OPB08 (right): From left to right populations: (M) marker 100bp, **1-** Himarë (natural population), **2**-Koplik (natural population) **3-** Leskovik (natural population), **4-** Lezhë (natural population), **5-** Shkodër (natural population), **6-** Skrapar (natural population), **7-** Himarë (commercial cultivar), **8-** Koplik (commercial cultivar), **9-** Leskovik (commercial cultivar), **10-** Lezhë (commercial cultivar), **11-** Shkodër (commercial cultivar), **12-** Skrapar (commercial cultivar).

The ability of the RAPD analysis to differentiate S.officinalis populations in the present investigation suggests that this technique can be very useful to the identification of genetic diversity among natural and cultivated populations of sage. Reproducibility of bands was good, and they were used to build clusters of similarity among the populations (Fig 5).

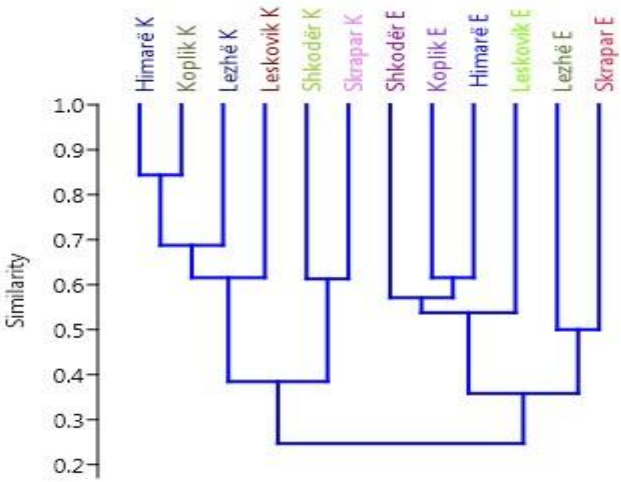


**Fig.5:** Dendrogram of polymorphic distances between analyzed samples, constructed by UPGMA method of PCoA software.

The dendrogram provided showed two main clusters. The first main cluster consisted of natural populations (populations to the right of the dendrogram) and the second cluster consisted of cultivated populations

(populations to the left of the dendrogram). Natural populations and commercial cultivars, according to *Jaccard* coefficient, had a similarity level approximately 25% where the diversity level is more present among natural populations compared to the cultivated ones. These two groups were joined together at about 0.250 genetic distance level.

Dendrogram cluster for the commercial cultivars showedtwo main clusters, one of them divided in 3 main sub-clusters and the other only of one sub-cluster, grouped in different genetic distances. The first sub-cluster consisted of Himara and Koplik populations, linked together at about 0.850 genetic distance. The second and the third sub-cluster consisted of Lezha and Leskovik population, respectively, which stay apart from the others in a genetic distance level approximately 0.70 and 0.63. The fourth one consisted of two populations, Shkodra and Skrapar, joined together at about 0.60 genetic distance level. Genetic distance levels of cultivated populations varied from 0.350 to 0.850. The high similarity level among cultivated populations compared to the natural populations shows that breeding programs do not explore the whole genetic variability of sage germplasms, as seen also in different studies from the Balkan and European countries (Echeverrigaray *et al*., 2006; Liber *et. al*., 2014). Reduction in the genetic variability of *Salvia officinalis* is one of the negative aspects of these kinds of breeding programs and it can affect seriously the genetic vulnerability of it or reduce sage selection profits, unless there are incorporated new sources of variation.

Dendrogram cluster for natural populations showed two main clusters, joined together at 0.350 genetic distance level. The first one was divided in four sub-clusters and the second one only in one. The first sub-cluster consisted of Shkodra population in a genetic distance level of 0.570. Likewise, the third sub-cluster consisted of Leskovik populations in a genetic distance level of 0.550. The third one consisted of two populations, Koplik and Himara, linked together at about 0.620 genetic distance level. The fourth sub-cluster consisted of two populations, Lezha and Skrapar, both of them in about 0.500 genetic distance level.

Natural populations showed significant diversity, while cultivated populations indicated a moderate diversity among one another.Our results indicate that RAPD markers are sufficiently informative to assess genetic diversity of Dalmatian sage populations. The application of molecular markers as RAPD markers, AFLP, RFLP and SSR markers have proven to be powerful enough in detecting variability even between very closely related individuals of the same aromatic plant species classified as ecotypes in previous studies (Bacu *et al*., 2005; Bacu *et al*., 2011; Papa *et al.,* 2016).

Sage has been cultivated intensively in North and South of Albania and in the Balkan countries like Croatia, Bosnia Herzegovina etc., for export purposes due to their essential oils (Kathe et al., 2003; Adhikari *et. al*., 2013;

Liber *et al*., 2014). This commercialization has reduced the appraisal of vegetation only in the GC definition of the essential oils content, which accompanies this product as a companion certificate to foreign markets (Kathe et. al., 2003; Kongjika et. al., 2005). Our results about the low diversity level between cultivated populations indicated that even in Albania among other Balkan and European countries, commercial gathering in the wildness has a negative impact on biodiversity conservation (Echeverrigaray *et. al*., 2006; Statovic *et. al*., 2012). Breeding programs based on detailed information on population genetic structure of aromatic and medicinal plants, such as *Salvia officinalis*, together with new accurate and appropriate *ex situ* conservation strategies would help the preservation and exploitation of these genetic resources in the country.

## 4. CONCLUSIONS

Genetic relationships between six natural populations and six commercial cultivars of *Salvia officinalis* L. is in the present paper evaluated. The RAPDs reactions showed that six out of ten random primers (OPA11, OPA08, OPA09, OPB08, OPB12, OPC15) were able to distinguish polymorphismsbetween *Salvia officinalis* populations.

Analysis of 12 samples in the study using 6 RAPD decameric primers generated a total of 63 fragments with an average of 60 polymorphic fragments per locus. The highest number of bands resulted in the analysis with the OPA11 primer, which generated 12 fragments, 11 of them polymorphic, while the lowest number of bands received was observed in the amplified samples with the OPA09 primer, which generated 9 fragments. The percentage of polymorphism generated for all primer varied from 80-100%.

PCoA analysis grouped populations into two major clusters, natural populations and commercial cultivars apart from each other.

Natural populations and commercial cultivars had a similarity of 25% where the level of diversity is more noticeable among the wild populations than the cultivated ones.

Among the commercial cultivars, those that showed greater similarity between them, respectively 85% according to *Jaccard* coefficient, are the cultivars of Himara and Kopliku.

All the natural populations reported significant, but moderate differentiation diversity among cultivated populations. The populations that showed greater genetic distance between them are natural populations of Leskovik and Shkodër, with about 55% according to *Jaccard* coefficient.

The high similarity level between commercial cultivars shows that commercial gathering in the wildness has a negative impact on biodiversity conservation of *Salvia officinalis* L.

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# CLASSIFICATION AND SELECTION OF SEED SOURCES IN KOSOVO

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## ABSTRACT

Qualitative seeds mean qualitative plants. Here, being aware about seed sources and to what extent they match the planting site and the purpose of the planting program is very important. Appropriate information about the location of seed sources would be useful. The following categories of seed sources ought to be defined and classified: i) seed collection zones, ii) identified and selected stands, iii) seed production areas and, iv) provenance seed stands. One of the purposes of classifying seed sources is to keep seed from different seed sources separate in order to be able to trace the ancestors of future plantings (Nikles and Newton1980). A seed source must consist of well distributed numerous trees on a given area making possible adequate inter-pollination so as to avoid increased risk of future inbreeding (Ballian 2011). Selection criteria were limited to a few phenotypic important traits estimated to have a relatively high heritability. The location of the seed sources of beech (*Fagus silvatica* **L.**), spruce (*Piceaabies* **Karst.**), fir (*Abies alba* **Mill.**), pine (*Pinus nigra* **Arn.**) were described and the boundaries were demarcated in the field. In default of information on origin careful estimation of the adaptability, growth and reproductive ability of the stand were carried out. It was important to select stands in which there is likely to be a significant genetic component in the phenotypic superiority. The first step taken in delineation was to produce an accurate map of the distribution of seed sources. The second step was to determine the original and large scale of populations. These main populations were then examined for lesser discontinuities resulting from soil types, mountain ridges and human interference.
***Keywords:*** seed sources, seed orchards, improved genetic quality

## 1. INTRODUCTION

Approximately 44.7% (481,000 ha) of Kosovo`s area is under forest cover (KNFI 2013) and the assessment, management and development this forestry estate to create both employment and investment opportunity is of paramount importance (Fig. 1). Assessment and analysis of potential areas for afforestation has been carried out in the framework of the FAO project (Support for the implementation of the Forestry Strategy in Kosovo). This report provides important data about the number of seedlings that should be produced, as well as the range of required species. The objectives of the National Afforestation/Reforestation Program (NARP) cannot be achieved without the production of high-quality forest seedlings of the appropriate species. The target for the forest nursery will be the production of forest seedlings suited to the site types predefined as being suitable for afforestation in Kosovo (Willan 1985).

Future production strategies should focus on the production of seedlings, from indigenous seeds, collected from national seed stands. Current production capacity should also be increased and this will require significant investment in both staff and mechanization. Indigenous seed stands selected for seed collection should cover all species outlined in the NARP. Future nursery production strategies will also need to consider the production of endangered species (Barner and Willan 1983).

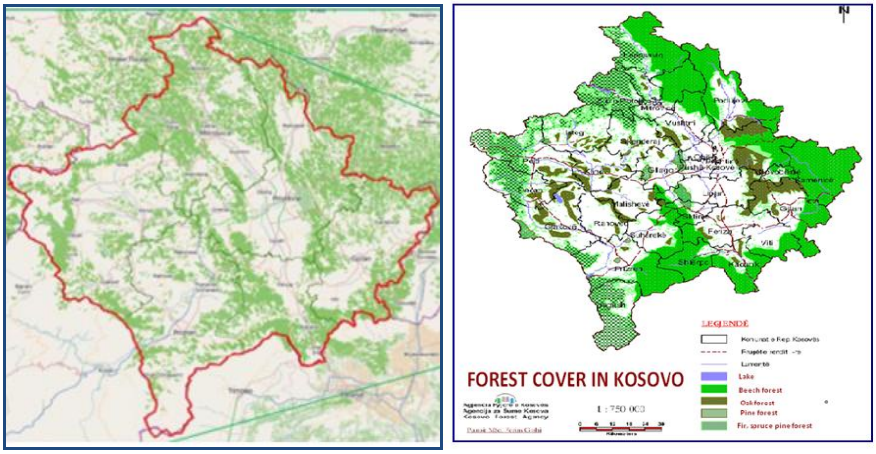



**Fig.1:** Location and forest cover of Kosovo.

Protecting the native genetic pool particularly of endangered plant species will ultimately make a significant contribution towards protecting the biodiversity of Kosovo. The advantages of focusing on native species are as follow: i) the genetic profile of the indigenous trees and shrubs which are of local origin will complement local growing conditions. The key principle should be the right tree in the right place, ii) planting trees that are better adapted to local site conditions will lead to a better plant survival rate thus avoiding the significant cost of replacing, iii) planting seedlings grown from imported seeds will affect the local genetic characteristics of certain species. This can manifest itself through delayed sprouting, delayed flowering and fructification. This in turn can disrupt the balance between the indigenous tree species and the wild life they support and, iv) using native seedlings will reduce transport costs.

The choice of seed source may be decisive for the success or failure of plantings (Willan 1984). To avoid mistakes the consumer must know which seed sources are available, what they are selected for and to what extent they match the planting site. To facilitate the choice of seed source for meeting the requirements it will be useful if publications are available on the location of seed sources and criteria for selection of the seed sources. The following categories of seed sources for collection of seed were defined and classified: seed collection zones, identified and selected stands, seed production areas, provenance seed stands (Gavranović *et al*., 2013). A seed source must consist of: i) trees of such an age and development that criteria for selection can be clearly judged and, ii) well-distributed numerous trees on a given area making possible adequate interpollination as to avoid increased risk of future inbreeding (Barner1975). Selection criteria were limited to a few phenotypic important traits estimated to have a relatively high heritability. The location of the seed sources in Kosovo [beech (*Fagus silvatica***L.**), oak (*Quercus s*.), spruce (*Piceaabies* **Karst.**), fir (*Abies alba* **Mill.**), pine (*Pinus nigra* **Arn.**)] were described and the boundaries of the sources were identified and demarcated in the field. A selected stand is a stand of trees superior to the accepted mean for the prevailing ecological conditions when judged by the selection criteria (OECD 1974). Based on the information collected at the regional offices of the Kosovo Forest Agency (KFA), based on questionnaires sent to the following offices, we organized on-site inspections of candidate stands to be selected for collection of seeds as planting material for future afforestations in Kosovo.

## 2. MATERIALS AND METHODS

Delineation process requires two important processes: i) the production of an accurate map of the distribution of seed sources in all Kosovo. It was important to select stands in which there is likely to be a significant genetic component in the phenotypic superiority and, ii) the determination of the original, large scale populations that were present. These main populations were then examined for lesser discontinuities resulting from soil types, mountain ridges and human interference.

**2.1. Selected stands**

**Definition**

A selected stand is a stand of trees superior to the accepted mean for the prevailing ecological conditions when judged by the selection criteria (OECD 1974). The stands are selected because of their phenotypic superiority in specified important traits. It is important to select stands in which there is likely to be a significant genetic component in the phenotypic superiority for instance, a stand should not be selected solely because it is growing well on an exceptionally good site. Selection criteria were limited to a few important traits. In many countries of temperate zone the following minimum requirements used by the OECD Scheme for the Control of Forest Reproductive Material are recognized (OECD 2013):

**Uniformity:** The stands must show normal degree of individual variation in morphological characters.

**Volume production**: Volume production of wood is normally an essential criterion for acceptance of selected stands. Volume production of wood must normally be superior.

**Wood Quality** shall be taken into account and in some cases, may become essential criterion.

**Form & Growth Habit**: The trees in selected stands must show particularly good morphological features, especially straightness and circularity of stem, favorable branching habit, small size branches and good natural pruning. The proportion of forked trees and with spiral grain should be low.

**Health and Resistance**: The trees in selected stands must in general be free from attack by damaging organisms and show resistance to adverse conditions of climate and site in the place.

**Geographic history**. The location of seed source must be described and the boundaries must be easily identifiable in the field, where necessary by demarcation. As regards origin, it must be carefully checked whether the stands are of local origin or introduced. Very often the information on origin is lacking.

**Genetic history must be carefully checked**.

Studying the artificial selection to which the stands have been subjected and clarifying whether the stand had derived from a seed lot representing a few parent trees only or from a larger population is very important. Measures must be taken to reduce the risk of contaminating pollen from foreign sources. Where isolation is difficult, a minimum size of 5 ha of the stand may be recommended and seed should not be collected along borderline of selected stand.

## 3. RESULTS

In the recent years, seedling production has been based on sowing seeds imported from abroad. Afforestation projects that have used these seedlings have often suffered from high levels of plant mortality, that can be attributed to a combination of factors which include: i) incorrect species selection in that the selected species is not compatible with local ecological conditions within the afforestation area, ii) lack of maintenance within the afforestation area post planting, iii) poor quality seed which was initially used to produce the seedlings and, iv) planting at lower altitudes where the species planted is in truth more suited to higher elevations.

Given the aforementioned deficiencies and the importance of the qualitative seeds, selecting native seed stands within Kosovo which have a defined, known phenotype and genotype and where the stand could be classed as being of "high quality" would be very important. Consequently, site inspection of forest stands was carried out involving a test for the conditions of the forest stands. Stands that did not fulfill one of the aforementioned criteria were excluded from the list of candidates for seed stands. Stands that fulfilled these criteria at first glance were accepted. However, measurements and observations for a final selection of seed stands ought to be made. Information about selected seeds stands is in the Table 1 reported.

**Table 1:** Selected seed stands in Kosovo

| Nr | Management Unit | Plot Nr. | Species | Area plot Ha | Area of seed stand |
|---|---|---|---|---|---|
| 1 | Bredhik | 33/1 | *Abies alba* L. | 37.86 | 6.10 |
| **2** | Koritnik | 15/1 | *Pinus heldreichii* **L.** | **90.30** | **5.70** |
| **3** | Bogaj | 32/1 | *Abies alba* **L.** | **23.59** | **5.50** |
| **4** | Ahishte | 31/1 | *Fagus sylvatica* **L.** | **15.20** | **5.50** |
| **5** | Deçan | 57/2 | *Picea abies* **Karst.** | **36.80** | **6.80** |
| **6** | Dragsh | 54/1 | *Pinus nigra* **Arn.** | **19.30** | **5.20** |
| **7** | Blinaja | 33/1 | *Quercus petrea* **L** | **21.50** | **9.70** |
| **8** | Gjilan | 24/1 | *Quercus cerris* **L.** | **25.40** | **6.80** |

### 3.1. Characteristics of selected seed stands

Dendrometric characteristics of the trees in the seed stands, by which the selection of plus seed stands was made is detailed in the Table 2.

**Table2.** Dendrometric characteristics of trees for the main species in the forest stand.

| a) Crown form | | | Number | % | b) Height of the crown | Number | % | c) Branching down | | | up | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Coniferous | | | 93 | 100 | | 93 | 100 | Category | Number | % | Number | % |
| Conical | | | 19 | 20 | 1/2 of Heigh | 09 | 10 | < 60 | 09 | 10 | 18 | 20 |
| Symmetric | | | 74 | 80 | 1/3 of Heigh | 16 | 15 | 60-90 | 18 | 20 | 18 | 20 |
| Flag | | | | | 1/4 of Heigh | 68 | 75 | >90 | 66 | 70 | 57 | 60 |
| d) Type of branching | | | Number | % | e) Full wood | Number | % | f) Right of the trunk | | | Number | % |
| Brushed | | | 19 | 20 | Weak | 00 | 00 | Weak | | | 05 | 05 |
| Brush | | | 09 | 10 | Good | 28 | 30 | Good | | | 14 | 15 |
| Flat | | | 65 | 70 | Very good | 56 | 60 | Very good | | | 56 | 60 |
| Indifferent | | | 00 | 00 | Great | 09 | 10 | Great | | | 18 | 20 |
| g) Trunk quality (Bifurcation) | | | Number 93 | % 100 | h) Branch diameter | Number 93 | % 100 | i) Number of branches in annual circles (for years of age) | | | Number 93 | % 100 |
| Low | | | 00 | 00 | Very large | 05 | 05 | 4 | | | 65 | 70 |
| On average high | | | 09 | 10 | Big | 05 | 05 | 5 | | | 18 | 20 |
| High | | | 5 | 05 | Medium | 09 | 10 | 6 | | | 09 | 10 |
| Does not exist | | | 79 | 85 | Thin | 74 | 80 | 7 | | | 00 | 00 |
| j) The distance between the annual down | | | up | | k) cleanliness of trees trunk | Number | % | l) Damages | | | Number | % |
| | Number | % | Number | % | | 93 | 100 | | | | 93 | 100 |
| 0.2 m | 09 | 10 | 18 | 20 | Weak | 09 | 10 | Strong | | | 00 | 00 |
| 0.4 m | 18 | 20 | 19 | 20 | Good | 28 | 30 | Medium | | | 00 | 00 |
| 0.6 m | 19 | 20 | 37 | 40 | Very good | 47 | 50 | Weak | | | 19 | 20 |
| 0.8 m | 47 | 50 | 19 | 20 | Great | 09 | 10 | Does not exist | | | 74 | 80 |
| m) Infections from disease and Insects | | | Number 93 | % 100 | n) Twisted trunk | Number 93 | % 100 | o) Rude bark finish | | | Number 93 | % 100 |
| Strong | | | 00 | 00 | Strong | 00 | 00 | High | | | 09 | 10 |
| Medium | | | 00 | 00 | Medium | 00 | 00 | Highly average | | | 19 | 20 |
| Weak | | | 05 | 05 | Weak | 09 | 10 | Low | | | 65 | 70 |
| Does not exist | | | 88 | 95 | Does not exist | 84 | 90 | | | | | |
| p) The structure of the bark | | | Number 93 | % 100 | q) The color of the bark | Number 93 | % 100 | r) Productivity | | | Number 93 | % 100 |
| Very cracked | | | 00 | 00 | Brownish-Reddish | 00 | 00 | Weak | | | 09 | 10 |
| Cracked | | | 09 | 10 | Gray easy | 65 | 70 | Good | | | 18 | 20 |
| With yarn | | | 18 | 20 | Dark gray | 28 | 30 | Very good | | | 65 | 70 |
| Smooth | | | 65 | 70 | | | | | | | | |

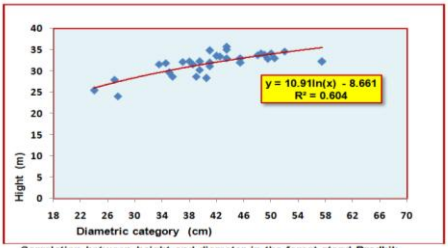
Here, a brief information on the inspected piles is provided along with the opinion at the end of each description, if accepted as seed stand. The information has been obtained based on the test surfaces of each seed cluster and is as follows:

1. Two pictures taken at the center of the test surfaces to show the quality of the seed stand.
2. Table on the distribution of trees by diametric categories in which forest stand.
3. Graphic on the correlation between height and diameter in the forest stand.
4. Graphic on the structure curve in relation to the diameter in the forest stand.
5. Graphic on the forest stand structure before and after thinning.

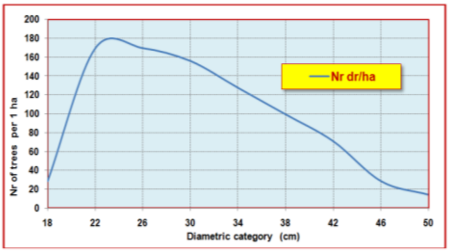
## 1. Seed Stand no. 1 Bredhik (*Abies alba* L.)

The distribution of trees by diametric categories in FS Bredhik

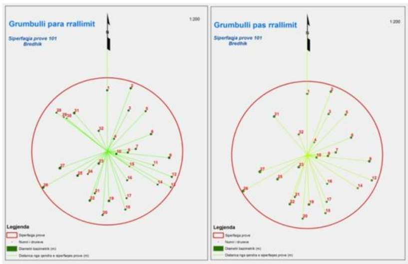
| Diametric category | Trees number/ha | Basimetric area m² /ha | The average size of tress | | |
|---|---|---|---|---|---|
| | | | $g_{mes}$ | $d_g$ | $h_g$ |
| 22 | 14 | 0.54 | | | |
| 26 | 28 | 1.50 | | | |
| 30 | 0 | 0.00 | | | |
| 34 | 57 | 5.14 | | | |
| 38 | 85 | 9.62 | 0.137 | 41.7 | 32.0 |
| 42 | 127 | 17.63 | | | |
| 46 | 42 | 7.05 | | | |
| 50 | 85 | 16.66 | | | |
| 54 | 0 | 0.00 | | | |
| 58 | 14 | 3.74 | | | |
| | 453 | 61.88 | | | |




Correlation between height and diameter in the forest stand Bredhik




The structure curve in relation to the diameter in the forest stand Bredhik

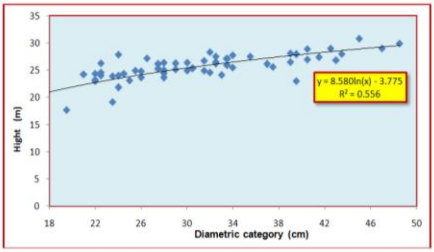



Forest Stand structure before and after thinning

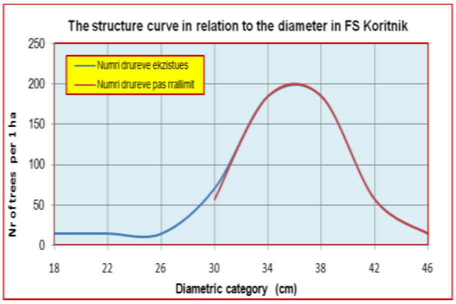
## 2. Seed Stand nr.2-Koritnik 2 (*Pinus heldeichii*)

The distribution of trees by diametric categories in FS Koritnik

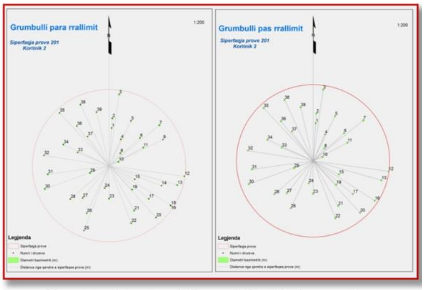
| Diametric category | Trees number/ha | Basimetric area m² /ha | The average size of tress | | |
|---|---|---|---|---|---|
| | | | $g_{mes}$ | $d_g$ | $h_g$ |
| 18 | 14 | 0.4 | | | |
| 22 | 14 | 0.5 | | | |
| 26 | 14 | 0.8 | | | |
| 30 | 71 | 5.0 | | | |
| 34 | 184 | 16.7 | 0.0985 | 35.4 | 21.2 |
| 38 | 184 | 20.9 | | | |
| 42 | 57 | 7.8 | | | |
| 46 | 14 | 2.4 | | | |
| | 552 | 54.4 | | | |




Correlation between height and diameter in the forest stand Koritnik




The structure curve in relation to the diameter in the forest stand Koritnik

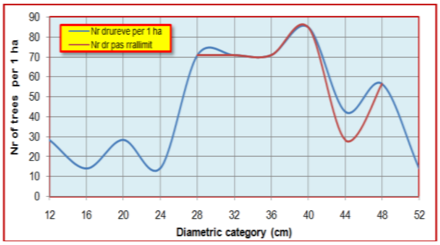



Forest stand structure before and after thinning operation

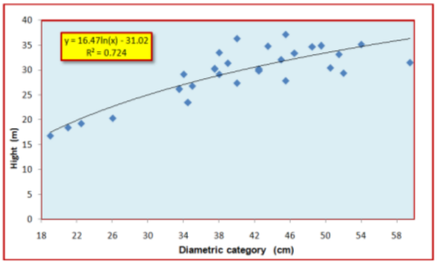
## 3. Seed Stand nr.3-BOGAJ *(Abies alba L.)*

The distribution of trees by diametric categories in FS Bogaj

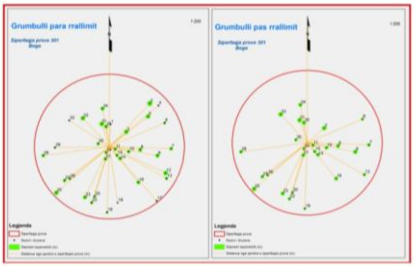
| Diametric category | Trees number/ha | Basimetric area m² /ha | The average size of tress | | |
|---|---|---|---|---|---|
| | | | $g_{mes}$ | $d_g$ | $h_g$ |
| 12 | 28 | 0.32 | | | |
| 16 | 14 | 0.28 | | | |
| 20 | 28 | 0.89 | | | |
| 24 | 14 | 0.64 | | | |
| 28 | 71 | 4.35 | | | |
| 32 | 71 | 5.69 | | | |
| 36 | 71 | 7.20 | 0.1004 | 35.8 | 26.4 |
| 40 | 85 | 10.66 | | | |
| 44 | 42 | 6.45 | | | |
| 48 | 57 | 10.24 | | | |
| 52 | 14 | 3.00 | | | |
| Totali | 495 | 49.73 | | | |




The structure curve in relation to the diameter in the forest stand Bogaj




Correlation between height and diameter in the forest stand Bogaj

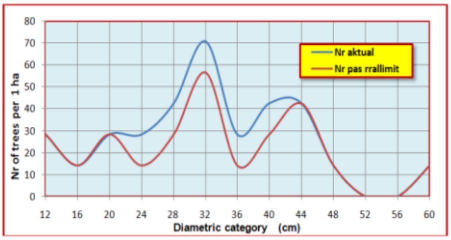



Forest stand structure before and after thinning operation in FS Bogaj

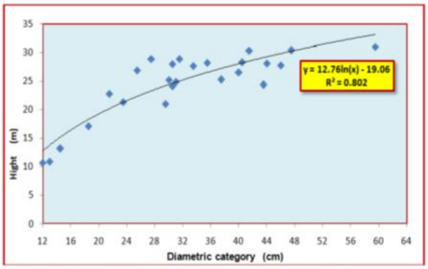
## <u>4. Seed Stand nr. 4-Ahishta (*Fagus sylvatica* L.)</u>

The distribution of trees by diametric categories in FS Ahishta

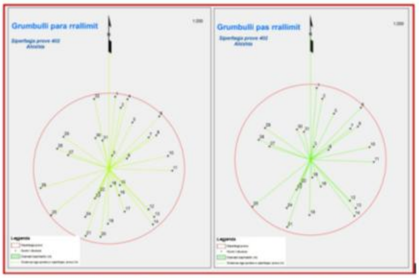
| Diametric category | Trees number/ha | Basimetric area m² /ha | The average size of tress | | |
|---|---|---|---|---|---|
| | | | $g_{mes}$ | dg(cm) | hg(m) |
| 12 | 28 | 0.32 | | | |
| 16 | 14 | 0.28 | | | |
| 20 | 28 | 0.89 | | | |
| 24 | 28 | 1.28 | | | |
| 28 | 42 | 2.61 | | | |
| 32 | 71 | 5.69 | | | |
| 36 | 28 | 2.88 | 0.091 | 34.1 | 26.0 |
| 40 | 42 | 5.33 | | | |
| 44 | 42 | 6.45 | | | |
| 48 | 14 | 2.56 | | | |
| 60 | 14 | 4.00 | | | |
| Totali | 354 | 32.29 | | | |




The structure curve in relation to the diameter in FS Ahishta




Correlation between height and diameter in the forest stand Ahishta

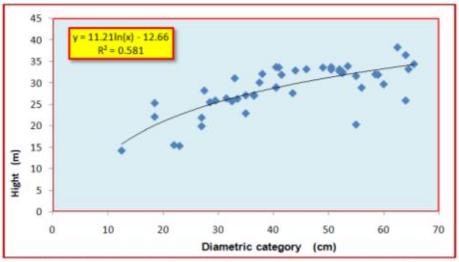



Forest stand structure before and after thinning operation in FS Ahishta

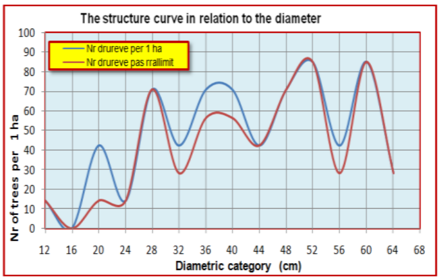
## 5. Seed Stand nr. 5- Deçan (Piceaabies)

**The distribution of trees by diametric categories in FS Deçan**

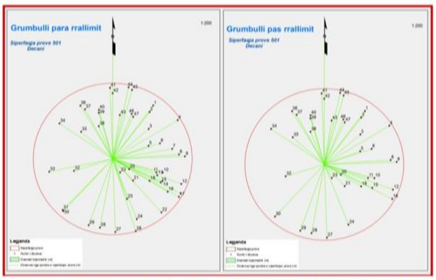
| Diametric category | Trees number/ha | Basimetric area m² /ha | The average size of tress | | |
|---|---|---|---|---|---|
| | | | $g_{mes}$ | dg(cm) | hg(m) |
| 12 | 14 | 0.160 | | | |
| 20 | 42 | 1.333 | | | |
| 24 | 14 | 0.640 | | | |
| 28 | 71 | 4.354 | | | |
| 32 | 42 | 3.412 | | | |
| 36 | 71 | 7.198 | 0.1572 | 44.8 | 29.97 |
| 40 | 71 | 8.886 | | | |
| 44 | 42 | 6.451 | | | |
| 48 | 71 | 12.796 | | | |
| 52 | 85 | 18.021 | | | |
| 56 | 42 | 10.450 | | | |
| 60 | 85 | 23.993 | | | |
| 64 | 28 | 9.099 | | | |
| Totali | 679 | 106.8 | | | |




Correlation between height and diameter in the forest stand Deçani




The structure curve in relation to the diameter in FS Deçan

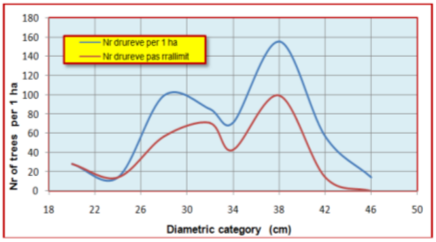



Forest stand structure before and after thinning in FS *Deçani*

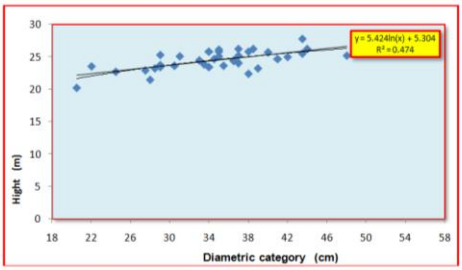
## 6. Seed Stand nr. 6-Dragash(*Pinus nigra)*

The distribution of trees by diametric categories in FS Dragash

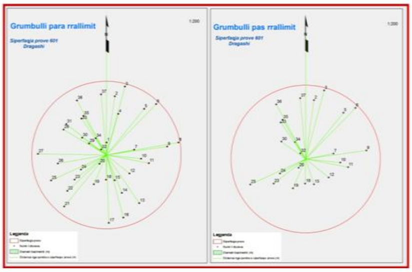
| Diametric category | Trees number/ha | Basimetric area m² /ha | The average size of tress | | |
|---|---|---|---|---|---|
| | | | $g_{mes}$ | dg(cm) | hg(m) |
| 26 | 57 | 3.0 | | | |
| 30 | 99 | 7.0 | | | |
| 34 | 142 | 12.8 | | | |
| 38 | 156 | 17.6 | 0.095 | 34.9 | 22.2 |
| 42 | 42 | 5.9 | | | |
| 46 | 14 | 2.4 | | | |
| Totali | 509 | 48.7 | | | |



The structure curve in relation to the diameter in FS Dragash



Correlation between height and diameter in the forest stand Dragash

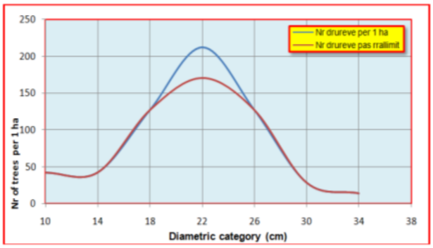


Forest stand structure before and after thinning in FS *Dragash*

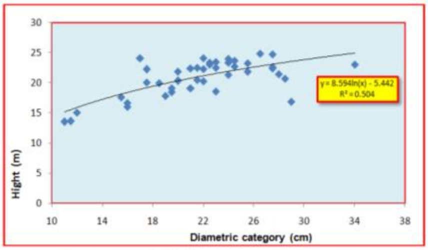
## 7. Seed Stand nr. 7- Blinaje (*Quercus petrea)*

The distribution of trees by diametric categories in FS Blinaja

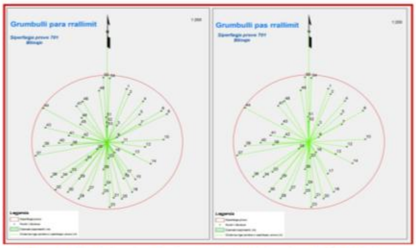
| Diametric category | Trees number/ha | Basimetric area m² /ha | The average size of tress | | |
|---|---|---|---|---|---|
| | | | $g_{mes}$ | dg(cm) | hg(m) |
| 10 | 42 | 0.33 | | | |
| 14 | 42 | 0.65 | | | |
| 18 | 127 | 3.24 | | | |
| 22 | 212 | 8.06 | 0.03757 | 21.9 | 21.1 |
| 26 | 127 | 6.76 | | | |
| 30 | 28 | 2.00 | | | |
| 34 | 14 | 1.28 | | | |
| Totali | 594 | 22.33 | | | |



*The structure curve in relation to the diameter in FS Blinaja*




Correlation between height and diameter in the forest stand Blinaja

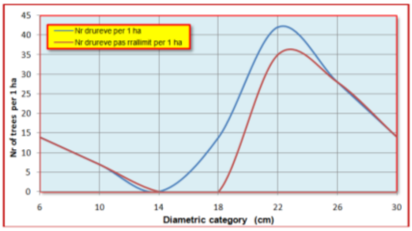



Forest stand structure before and after thinning in FS Blinaja

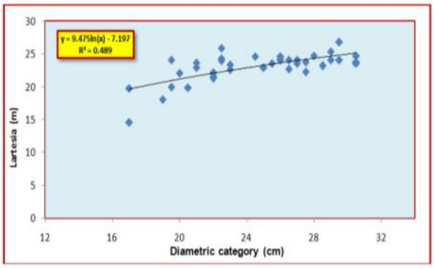
## 8. Seed Stand nr. 8- Gjilan(*Quercus cerris)*

The distribution of trees by diametric categories in FS Gjilani

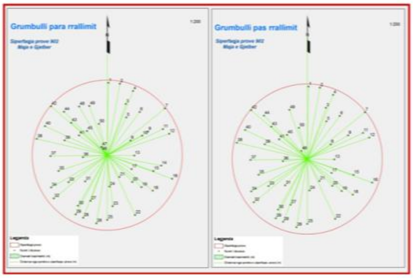
| Diametric category | Trees number/ha | Basimetric area m² /ha | The average size of tress | | |
|---|---|---|---|---|---|
| | | | $g_{mes}$ | dg(cm) | hg(m) |
| 12 | 14 | 0.16 | | | |
| 16 | 36 | 0.71 | | | |
| 20 | 135 | 4.24 | | | |
| 24 | 149 | 6.74 | 0.046 | 24.1 | 23.0 |
| 28 | 149 | 9.18 | | | |
| 32 | 50 | 4.00 | | | |
| Shuma | 547 | 25.02 | | | |




*The structure curve in relation to the diameter in FS Gjilan*




Correlation between height and diameter in the forest stand Gjilani




Forest stand structure before and after thinning in FS Gjilani

## 4. CONCLUSIONS

Natural forests are the base material for all seed sources and must generally be expected to represent an “***average***” quality (tab. 4). Trees growing in natural forests are expected to show a high degree of adaptation to the prevailing environmental conditions of the site. Pollination is usually effective in natural forest. However, due to seed dispersal, there is in natural forests a risk that trees form family groups i.e. neighboring trees are related or in extreme cases inbred. This will normally be broken in next generation plantation seed source. To assure genetic diversity is advised that seeds from natural forest be collected from widely separated trees. Seed sources in natural forests should accordingly typically be larger than plantations.

Natural forests consist of indigenous species, which has spontaneously generated themselves on the location for many generations. Natural forests include both edaphic and climatic climax types and pioneer forests. Natural forests can be more or less influenced by culture, e.g. by logging/regeneration techniques, but the forests must not have been subject to regeneration by sowing or planting. However, enrichment planting and sowing using local material will still be considered natural forest.Selection of mother trees/seed trees in natural forest usually gives little, if any, genetic gain since environment and age difference conceal or veil possible genetic variation.

**Table 4:** Key Dendrometric characteristics of the seed stands

| **Key Dendrometric characteristics** | **1.Bredhik** Abies alba | **2.Koritnik** Pinus heldreichii | **3.Bogaj** Abies alba | **4.Ahishta** Fagus sylvatica | **5.Deçan** Picea abies | **6.Shtime** Pinus nigrae | **7.Blinaja** Quercus petrea | **8.Gjilan** Quercus cerris |
|---|---|---|---|---|---|---|---|---|
| Average diameter (cm) | **41.7** | **35.4** | **34.1** | **34.1** | **44.8** | **34.9** | **21.9** | **24.1** |
| Average height (m) | **32.0** | **21.2** | **26.0** | **26.0** | **29.9** | **22.2** | **21.1** | **23.0** |
| Trees number per ha | **453** | **552** | **495** | **354** | **679** | **509** | **594** | **547** |
| Basimetric area ($m^2$/ha) | **61.9** | **54.4** | **49.7** | **32.3** | **106.8** | **48.7** | **22.3** | **25.1** |
| Stand volumme ($m^3$/ha) | **774** | **664** | **648** | **870** | **507** | **491** | **518** | **586** |
| Age (years) | **97** | **117** | **90** | **136** | **105** | **74** | **84** | **78** |

However, relatively 10 evenaged natural forests exist for example as pioneer forest after land slide, burning, shifting cultivation or other disaster events. Many types of natural forests are nowadays protected forests bounded by cultivated land. Even if the total distribution of the forests may be large, some individual species could have a limited distribution within the forest. Natural forests are typically used to ‘mobilise’ the genetic reserve. Before undertaking the establishment of seed producing tree stands it is very important to first understand seed quality, since good quality seeds are needed to generate good quality trees. Bad quality seeds will produce trees with bad traits and/or that grow poorly. When dealing with seed quality there are three factors that have to be taken into consideration: genetic quality, physical quality and physiological quality:

The ***physical quality*** of seeds includes their size, color, age and seed coat condition. Cracks, damages or the presence of pests or diseases may all negatively affect germination.

The ***physiological quality*** of seeds includes the seed purity, moisture content and integrity of tissues, all of which will influence germination capacity.

The ***genetic quality*** is determined by the information contained in the genes within the seeds and is therefore inherited. High genetic diversity is a decisive factor in the success of any tree-planting project. Seeds of good genetic quality that are grown in the right environment and managed in the right way usually generate trees with desirable traits. It was very important to decide ahead of time on the right tree species to grow in the stand, since each species provides different products and is adapted to grow under specific ecological conditions.

Additionally, the species selected should come from an area with similar environmental conditions as to those at the site where the stand will be established. Finally, if the species selected is crosspollinating and is new to the area, it is important to ensure that the right pollinators are present.

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# TREATMENT OF SKIN CANCER WITH ALTERNATIVE MEDICINE METHOD

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## ABSTRACT

More people are diagnosed with skin cancer each year in the world. Albania has a high incidence rate of people diagnosed with this disease in the last three decades. In the present paper, 260 patients in total diagnosed with skin cancer underwent medical treatment using NILS (extracts of some Albanian plants *Allium sativum*, *Juglans regia* and *Laurus nobilis)* at the ALMS "STROKA" Clinic from June 2015 to June 2017. The age of the patients varied from 9 to 99 years old. Diagnosis was established based upon the signs and symptoms, dermoscopy and biopsy. The treatment period varied from 1 to 6 months, depending on patients' regenerative abilities. On average, the treatment period was 2 months until complete healing of the lesion and consisted of 7-10 sessions as recommended by the treating physician and depending on the tumor mass characteristics. Because it is an open medication, since the first sessions, we could see a shrinkage and reduction of the cutaneous lesion size, up to the total disappearance of the lesions at the end of the treatment. At this point, the treatment with NILS was interrupted, because the natural layers breakaway ended, and the process of regeneration of normal skin started. Once the treatment period ended, the patients were advised to be under control on a regular basis for a period of 3 years.
***Keywords***: skin cancer, diagnosis, treatment, medicinal plants, alternative medicine

## 1. INTRODUCTION

There are two main types of skin cancer - non-melanoma and melanoma - along with some much rarer types. Non-melanoma is more common, and it's much less likely to spread. The most common non-melanoma tumours are basal cell carcinoma and squamous cell carcinoma. Melanoma of the skin is the 19th most commonly occurring cancer in men and women. There were nearly 300,000 new cases in 2018. Non-melanoma skin cancer (NMSC) is the 5th most commonly occurring cancer in men and women, with over 1 million diagnoses worldwide in 2018 (https://www.wcrf.org/dietandcancer/cancer-trends/skin-cancer-statistics).

The number of skin cancer affected people and in particular people suffering from Malignant Melanoma (MM) has significantly increased over the years even in Albania. The European countries with the lowest estimated incidence in melanoma were Moldova, Bosnia and Herzegovina and Albania, with nearly 3.4 per 100,000. The European countries with the highest estimated mortality were Norway, Slovenia and Sweden, compared with the European average of 2.3 deaths per 100000 PY. Bosnia and Herzegovina, Malta and Albania had 0.7 deaths per 100,000 with the lowest estimated mortality rates from MM among the European countries in 2012 (https://encr.eu/sites/default/files/factsheets/ENCR_Factsheet_Malignant_Melanoma_2015.pdf.).

Populations at risk are those with fair skin freckling, tendency to sunburn with high number of sunburns in life, red/blond hair, or having a high number of nevi (or moles) or any number of atypical nevi, or a history of organ transplantation, and/or taking immunosuppressive medication regimens (Vinzón*et al*., 2014). The high incidence of ultraviolet radiation and increased exposure to it by the population during childhood and adolescence, through sunburns and clothes exposing large body areas may be one of the factors, as well as the socio-economic level (Pereira*et al*., 2015). Patients at high risk may include those having a previous history of cancer or other risk factors, such as having atypical nevi or many common nevi, having a family or personal history of skin cancer, occupational exposure, or intense exposure such as indoor tanning (Watson*et al*., 2017).

In comparison to UVA radiation, UVB is more lethal and acts as a complete carcinogen (Singh*et al*., 2014). NMSC can be caused by ionizing radiation arising in sites of chronic radiation damage, chronic inflammation, hydrocarbons(tar), and chronic ingestion of inorganic arsenic; these tumors can be much more aggressive than those associated with UVR or HPV (Fitzpatrick*et al*., 2005). First, we are targeting the three primary factors needed to result in reduced risk of serious skin cancers: i) patient skin self-examinations; ii) physician full-body skin exams; and iii) rapid access to dermatologist evaluation of worrisome lesions (Daniel*et al*., 2015).

Cutaneous epithelial (NMSC) are the easiest of all cancers to diagnose and treat (Fitzpatrick*et al*., 2005). The clinical diagnosis of skin cancer is based on visual examination followed by biopsy of suspicious lesions (Lui *et al*., 2012). The search to improve our clinical diagnostic accuracy for identifying skin cancer and to minimize unnecessary skin biopsies has led to the development of non-invasive imaging techniques, where RCM features of common melanocytic and non-melanocytic skin neoplasms such as melanoma, actinic keratosis/squamous cell carcinoma, basal cell carcinoma, and nevi have been well defined and show good correlation with dermoscopic and histopathologic findings (Ahlgrimm-Siess*et al*., 2018).

Equally important, we will attempt to narrow the interval from first detection of suspected skin cancer to treatment through the added dimension of teledermoscopy, which provides highly accurate preliminary information about the morphology of a lesion and can, in turn, lead to expedited care if needed (Daniel *et al.*, 2015). To compare Raman spectroscopy in distinguishing: i) malignant and premalignant lesions from benign disorders, ii) melanomas from benign pigmented skin lesions, and iii) melanomas from seborrheic keratoses with other non-invasive diagnostic techniques as well as with clinical diagnosis by visual examination, we calculated skin biopsy ratios at sensitivity levels of 90%, 95%, and 99%, respectively (Lui *et al*., 2012). In vivo RCM has been shown to increase the accuracy of non-invasive diagnosis of common skin neoplasms, and is a valuable adjunct to dermoscopy, particularly in cosmetically and functionally sensitive areas such as the face or the genital area (Ahlgrimm-Siess*et al*., 2018). An Australian study found that the clinical diagnosis of skin cancers and precancers was associated with a sensitivity of 63.9% for BCCs, 41.1% for SCCs, and 33.8% for malignant melanomas (Lui *et al*., 2012). Dermoscopy has limited utility when it comes to the diagnosis of pink, erythematous, non-pigmented skin lesions (Ahlgrimm-Siess*et al*., 2018). With dermoscopy, the sensitivity to diagnose melanoma is 85 %and better compared to 65 to 80% when the technique is not used (Johr*et al*, 2015). Dermoscopy helps to differentiate melanocytic from nonmelanocytic skin lesions to differentiate benign from malignant skin lesions (Johr*et al*.,2015). Many melanomas may appear banal and therefore be overlooked, whereas benign pigmented lesions can sometimes show clinically suspicious features on visual examination and therefore be unnecessarily biopsied (Lui *et al*., 2012).

The two principal NMSCs are basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) (Fitzpatrick*et al*., 2005). The analysis presented here focuses specifically on those diagnostic classes of skin lesions that characteristically give rise to patient and physician concern over skin cancer, including: i) malignancies and premalignancies that require treatment: malignant melanoma, SCC, BCC, and actinic keratosis, and ii) benign conditions that can visually mimic skin cancer: seborrheic keratosis, atypical nevi, melanocytic nevi (junctional, compound, and intra-dermal), and blue nevi (Lui *et al*., 2012). Metastatic cancer to the skin is characterized by solitary or multiple dermal or subcutaneous nodules, occurring as metastatic cells from a distant non-contiguous primary malignant neoplasm, that are transported to and deposited in the skin or subcutaneous tissue by hematogenous or lymphatic routes, or by contiguous spread across the peritoneal cavity or other tissues (Fitzpatrick*et al*., 2005).

The treatment of skin cancer includes some options, such as freezing, excisional surgery, Mohs surgery, curettage and electrodesiccation or cryotherapy, radiation therapy, chemotherapy, photodynamic therapy and biological therapy.260 patients with MM and NMSC have undergone the treatment with an alternative method which is inherited and enriched in some generations by using NILS, the herbal solution that has come to be perfected from generation to generation to these days.

The present paper aims at evaluating the results obtained by applying the alternative medicine to patients diagnosed with skin cancer.

## 2. MATERIALS AND METHODS

In the present paper 260 patients in total diagnosed with skin cancer underwent medical treatment using NILS (extracts of some Albanian plants *Allium sativum*, *Juglans regia* and *Laurus nobilis)* at the ALMS “STROKA” Clinic from June 2015 to June 2017. 185 cases were reported with Basal Cell Carcinoma (BCC), 65 cases with Squamous Cell Carcinoma (SCC), 9 cases with Malignant Melanoma (MM) and 1 case with Porocarcinoma. The age of the patients varied from 9 to 99 years old. Diagnosis was established based upon the signs and symptoms, by dermoscopy and biopsy. 14,6% of the patients were diagnosed through biopsy at the Centre hospitalier regional universitaire de Besançon, University Medical Centre of Tirana "Mother Teresa", University Clinical Centre of Kosovo, etc. and their treatment was carried out at these hospitals centers but were referred to our clinic because of relapses.

The treatment was performed locally by applying the solution on the tumor surface. The treatment consisted in dying the lesion by using extracts of some Albanian plants (Allium sativum, Juglans regia and *Laurus nobilis*) called NILS. NILS has fluid consistency and brown color. Diagnosis was established based upon the signs and symptoms, dermoscopy and biopsy. The treatment period varied from 1 to 6 months, depending on patients’ regenerative abilities. On average, the treatment period was 2 months until complete healing of the lesion and consisted of 7-10 sessions as recommended by the treating physician and depending on the tumor mass characteristics. This treatment was applied once a day and took at least 10 to 30 minutes in large-scale lesions. The first week had the most frequent sessions (3-4 times a week), followed by the second week (2-3 times a week), and after that the number of NILS treatment sessions was reduced or interrupted, because the natural layers breakaway ended, and the process of regeneration of normal skin started. Cases were accompanied by photos in different phases of the treatment from the beginning to the end. In general, tumor diameter was > 5 mm (from 7- 70 mm and the thickness up to the level of the skin was 3-30 mm) and mostly ulcerated (Photo1, 2, 3).

**Photo 1** Lip lesion treatment phases of the same patient.

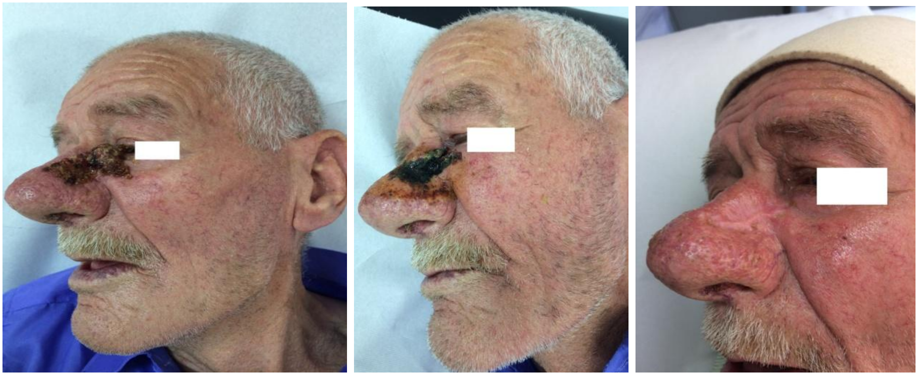


**Photo 2.** Nose lesions treatment phases.

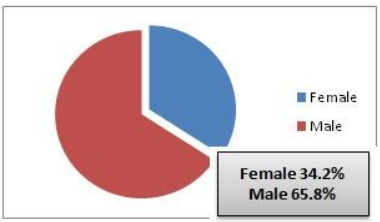
**Photo 3.** Chest lesions treatment phases.

## 3. RESULTS

Shrinkage and reduction of the cutaneous lesion size was reported in the first sessions and the total disappearance of the lesions at the end of the treatment. Neither undesirable side effects on elderly patients, nor those with chronic cardiovascular, pulmonary, gastrointestinal, metabolic and endocrine disease and no toxic effect to any patient was reported. Local pain was caused by the product applied over mostly ulcerated wounds, headache in sporadic cases, but associated with the use of 100 mg of aspirin when the lesion was from the level of the upper lip and above, swelling when extended sessions were conducted on the area around the eye, and sneezing when the patient was allergic to the herbal content of the product, but which was transient and ended at the end of the treatment. These undesirable side effects were observed on a limited number of patients. No toxic effect from the local application of NILS has ever been observed. Table 1 reports skin cancer classification based on age group. Graphic 1 plots skin cancer classification based on gender. Table 2 reports skin cancer treated for the first time and skin cancer treated earlier with surgery and / or radio therapy.

**Table 1** Skin cancer classification based on age group

| Age group | Percentage of patients |
|---|---|
| 0-20 years | 0.4 % |
| 21-40 years | 2.7% |
| 41-60 years | 27.3% |
| 61-80 years | 56.5% |
| >80 years | 13.00% |




**Graph 1:** Skin cancer classification based on gender

**Table2**. Skin cancer treated for the first time vs. skin cancer treated earlier with surgery and / or radio therapy

| | |
|---|---|
| Treated for first time in our clinic | 85.4% |
| Treated before with surgery or/with radiotherapy | **14,6%** |

**Table3.**Skin cancer classification based on affected area

| Location of the lesion | % |
|---|---|
| Head and neck | 80 % |
| Body and extremity | 20% |

## 4. DISCUSSIONS

Cancer is a major burden of disease worldwide. Positive results and commitment to providing the highest standard of care to the patients make the alternative medicine a success story. Cases of treatment failure are reported, however, due to lack of cooperation and unfollowing of antiseptic rules from our patients during home treatment.

Dermatologists, surgeons and skin cancer doctors are faced with an epidemic of skin cancer in Australia and New Zealand, given the need to reduce unnecessary surgery as well as associated costs, have turned their focus to topical applications to deal with skin cancer (Tampa 2016).

Ingenolmebutate, hypericin, coffee, tea, black salve, bloodroot, paclitaxel, and beta-carotene have been studied for their effects on NMSC in humans or have been reported to be used in humans with BCCs and SCCs. Clinical trials assessing the effectiveness of ingenolmebutate on BCCs and SCCs and case reports with patients using paclitaxel for BCCs suggest efficacy of these agents for treating NMSCs in humans, black salve and bloodroot were reported to be more harmful than therapeutic (Millsop*et al.*, 2013).

5-fluorouracil has been around since the 1960s and it acts as an antimetabolite, Diclofenac, an NSAID, acts by downregulating cyclooxygenase enzymes and increasing apoptosis. Topical diclofenac 3 % gel in 2.5 % hyaluronic acid acts by reducing dysplastic keratinocytes in cancerous lesions. Ingenolmebutate has a dual action: the induction of rapid cellular death in the treated area, followed by an inflammatory response, able to eliminate residual cells. In many studies,imiquimod (INN) has shown itself effective against skin cancers and pre-cancerous lesions. Therefore, while imiquimod has been added to our topical armamentarium with respect to skin cancer management, caution must be exercised in prescribing this treatment, and it is especially important to follow-up patients regularly. In recent times Imiquimod has been shown to paradoxically cause tumours, or more precisely tumors have been reported on bodily sites of treatment. Some prevailing topical treatments include 5-fluorouracil, diclofenac sodium, topical photodynamic therapy (PDT) with 5-aminolevulinic acid (ALA) treatment site. The reports of Imiquimod causing aggressive SCC, or in one case, an invasive melanoma arising at the site of topical Imiquimod use, suggest follow-up (Tampa 2016).

**Skin Cancer**

In the present paper, 260 patients in total diagnosed with skin cancer (from 7- 70 mm in diameter and the thickness up to the level of the skin was 3-30 mm and mostly ulcerated) underwent medical treatment involving NILS (extracts of some Albanian plants *Allium sativum*, *Juglans regia* and *Laurus nobilis)* at the ALMS "STROKA" Clinic from June 2015 to June 2017.

In most cases, patients underwent the treatment when the size of the lesion became worrying, it started blooding or draining purulent secretions, or after previous unsuccessful treatments. From the first sessions of the treatment, the lesion significantly reduced in size, bleeding and drainage stopped. In the end, the lesion disappeared.

Over 3 million new cases of skin cancer are diagnosed in the US annually (Diaoand Lee 2014). Skin cancer is an increasingly important global public health problem (McWhriter and Hoffman-Goetz 2006). Epidemiological data show a continuing increase in the incidence of non-melanoma and melanoma skin cancers (Schalka*et al*., 2014). Incidence, however, varies by latitude and altitude, with regions closer to the equator and higher in altitude generally having higher rates of skin cancer (Kasparian *et al.*, 2012). The skin is considered as the largest organ in the human body, and it protects against heat, sunlight, injury, and infections (Penta *et al.*, 2017). Actinic keratoses (AK) are precancerous lesions that increase the risk of skin cancer in patients with chronic sun damage and/or immunosuppression (Maytin*et al*, 2018). Genetic factors strongly influence the risk of skin cancer (Watson *et al*, 2016). Viruses are another agent which transforms keratinocytes by activation of cancer-promoting genes (Singh *et al*.,2014). The skin is the most frequent location of primary malignant neoplasms (Duarte *et al*., 2018). Skin cancers can be classified depending on the involved cell type: keratinocytes and melanocytes (Gálvez*et al*.,2018).

Skin cancer (including malignant melanoma, squamous cell carcinoma, and basal cell carcinoma) is a common disease in all European-derived populations and has shown increases in incidence over the last century (Kasparian *et al*.,2012).

The pathogenesis of skin cancer requires both genetic and nongenetic molecular alterations (Ming *et al* 2014). Latinos have the highest rate of skin cancers among U.S. minorities (Rodríguez *et al*.,2018). Skin cancer among women under 40 years of age has been observed to be increasing in both cases with single and multiple lesions and those, malignant skin neoplasms were the most frequent in men (Pereira *et al*, 2015). However, studies analysing specific risk factors for skin cancers, such as skin complexion, eye colour, and skin response to sun exposure are limited (Gadalla*et al*, 2017).

The lifetime cost of the 150,000 incident cases of skin cancer diagnosed in NSW in 2010 is estimated at $536 million ($44,796 per melanoma and $2459 per non-melanoma) (Doran *et al.,* 2015).

The pathogenesis of skin cancer requires both genetic and nongenetic molecular alterations (Ming *et al*., 2014). Cellular secretion is an important mediator of cancer progression (Almiron*et al*.,2018). The incidence of skin cancer increases with age (Doran *et al.,* 2015).

**Non-Melanoma Skin Cancer**

185 cases were reported with basal cell carcinoma (BCC), 65 cases with squamous cell carcinoma (SCC), 11 out of which suffered from lip cancer. The patients with a single lesion were rare. In most of the cases the patients had at least 2 or 3 lesions. The disease had generally advanced or advanced years after lesion’s appearance and the standard treatment proved to be ineffective. In these cases, the patients had an extended follow–up with subsequent positive results. Seven non-melanoma skin cancer including one with lip cancer resulted in NILS treatment failure.

Basal cell carcinoma (BCC) is the most common form of skin cancer followed by squamous cell carcinoma (SCC) (Doran *et al*, 2015).

A long-standing, slow-growing and painless tumor should be suspected for BCC (Katsambas and Lotti 2010). Basal cell carcinoma (BCC) is a skin cancer derived from nonkeratinizing cells that form the basal layer of epidermis (Rigel *et al*., 2011).

The most common are basal cell carcinoma and squamous cell carcinoma (Diaoand Lee 2014). These categories are also widely known as non-melanoma skin cancer (NMSC), with a higher incidence rate, and melanoma skin cancer (MSC), with a higher mortality rate, respectively (Gálvez*et al*., 2018). Risk for BCC varied based on type of skin cancer in relatives, age of onset in relatives, and degree of affected relatives (Berlin *et al*., 2015). Squamous cell carcinoma (SCC) and basal cell carcinoma (BCC) account for over 90% of skin cancers in organ transplant patients, and affect over 50% of white transplant recipients (Diao and Lee 2014).

Cutaneous squamous cell carcinoma (SCC) is a malignancy arising from epithelial keratinocytes (Rapini 2005). Actinic keratoses (AK) are very common premalignant skin lesions (presquamous cell carcinoma) in patients with chronic sun damage (Maytin*et al.*, 2018). The typical lesion (of SCC) is an indurated, firm, raised, skin-colored or pink to red nodule, whose growth can progress rapidly when extensive ulcerative necrosis occurs (Giannotiand De Giorgi 2010). Squamous cell carcinoma is the second most common type of skin cancer (Singh *et al*., 2014). Margins of induration are usually poorly defined (Giannotiand De Giorgi 2010).

Squamous cell carcinoma (SCC) of the skin is the most aggressive form of non-melanoma skin cancer (NMSC), and is the single most commonly diagnosed cancer in the U.S., with over one million new cases reported each year (Yin*et al*., 2006). Oral squamous cell carcinoma (OSCC) remains a global health problem (Chen *et al*., 2018). It is easily treated when detected early, but in a small percentage of cases this cancer has metastasis potential (Singh *et al,* 2014). The initiation and development of oral SCC is caused by a combination of genetic alterations, environmental risk factors and viral infection (Zhao *et al*., 2018). SCC was associated with a 24% risk increase in women but little to no association in men; BCC was associated with a 25% risk increase in women and 17% in men (Rees *et al*., 2018).

**Malignant Melanoma**

Only 9 cases were diagnosed and treated with malignant melanoma,8 of which were ulcerated,but without palpable lymph node. In Malignant Melanoma the treatment was more painful,but NILS absorption was more rapid and lesion reduction was more noticeable. Due to the depth treatment,the recovery time was extended,especially in the scalp and abdominal skin.

Melanoma, a subtype of skin cancer that can be fatal if the disease is not detected and treated at an early stage, is the most common cancer among those aged 25–29 years and the second most common cancer among adolescents and young adults aged 15–29 years (Diaoand Lee 2014). The early diagnosis of skin melanomas is usually determined by using ABCDE signs (Gálvez*et al.*, 2018). Melanoma is the deadliest form of skin cancer that is derived from the uncontrolled growth of melanocytes derived from neural crest cells (Shin *et al.*, 2018). Malignant melanoma or black skin cancer originates from the transformation of melanocytes or naevus cells (Baldaand Starz 2010). Melanocytes, cells found in the basal layer of the skin and give the skin and eyes their colour, are genetically programmed to produce a specific amount of melanin (Watson *et al.*, 2016). Melanoma was the most costly skin cancer diagnosis (Qureshi 2011). Although melanoma accounts for only 4% of diagnoses of skin cancer, it accounts for 80% of skin cancer-related deaths (Qureshi 2011). In addition to skin tone, melanoma risk is associated with total nevi count (Watson *et al,* 2016). A comprehensive meta-analysis has demonstrated that increased sunburns from childhood, adolescence, and adulthood all increase the risk of melanoma (Diaoand Lee 2014).

For participants with no history of melanoma, the likelihood of engagement in SSE (skin self-examination) at least once per year increased amongst those with one or two, or more than five moles greater than 6 mm in diameter, as well as those with greater perceived risk of developing skin cancer, perceived severity of skin cancer, perceived benefits of SSE, self-efficacy, and social norms (Kasparian *et al.*,2012). Another physical feature that is an independent risk factor for melanoma occurrence is the high number of melanocytic nevi and/or the presence of atypical nevi (Diaoand Lee 2013).Skin melanoma is less common and is associated with high metastasis and mortality rates (Pereira*et al.*,2015). Malignant melanoma accounts for less than five percent of skin cancer cases, yet it represents the vast majority of skin cancer deaths in Australia (Doran *et al*,2015). Family history of melanoma; having one first-degree relative (FDR) doubles one's risk, and three or more FDRs increases risk by 35–70 times (Diao*et al*,2013). Previous personal history of skin cancer; with melanoma patients having an elevated risk of 4%–9% for developing another primary melanomahas been reported in (Diaoand Lee 2013). Skin melanoma was most common among women (single or multiple lesions) (Pereira *et al*, 2015). Light skin pigmentation, blond or red hair, blue or green eyes, and prominent freckling tendency are phenotypic features associated with an increased risk of melanoma (Diaoand Lee 2013). Nearly half of the patients with stage IV melanoma develop brain metastases (Rodenburg*et al.*, 2016).

***Aetiology and prevention***

More than one million new skin cancers are diagnosed yearly in the United States creating the need for effective primary and chemo-preventive strategies to reduce the incidence, morbidity, and mortality associated with skin cancer (Einspahr*et al.*,2002).

Diets high in fruit, vegetables,grains and legumes appear reduce the risk of a number of diseases, including cancer (Balch and Balch 2000). Poor diet and obesity, smoking and genetics includes 70 % of cancerous factors (Balch and Balch 2000).

Physical activity is associated with a nearly 40% reduction in the cancer risk associated with chronic diseases (Huakang*et al*, 2018).

Supplementation with vitamin D did not result in a lower incidence of invasive cancer or cardiovascular events than placebo (Keaneyand Rosen 2017; Manson *et al.*, 2018). Supplementation with O−3 (also called omega-3) fatty acids did not result in a lower incidence of major cardiovascular events or cancer than placebo (Manson*et al*., 2018). Although opinions are controversial, in two other studies higher vitamin D concentration was associated with lower risk of total cancer (Budhathoki*et al.*, 2018) andhigher intake of marine O−3 fatty acids has been associated with reduced risks of cardiovascular disease and cancer in several observational studies (Manson *et al.*, 2018).

In general, participants across groups reported that skin cancer was preventable (Rodríguez*et al*,2018). Considerable efforts have been made to identify the phytochemicals which may possibly act on one or several molecular targets that modulate cellular processes such as inflammation, immunity, cell cycle progression, and apoptosis (Singh *et al.*,2014). Two most studied phytochemicals such as sulforaphane and tea catechins/epicatechins have epigenetic regulations in the prevention and therapeutics of skin cancer (Penta *et al.*,2017). Phytochemicals have antioxidant, antimutagenic, anticarcinogenic, and carcinogen detoxification capabilities, thereby considered as efficient chemo-preventive agents (Singh *et al.*, 2014). The use of bioactive dietary phytochemicals against various diseases including cancers remains attractive in the field of dietary cancer prevention and therapy(Penta *et al.*, 2017).

A diet rich in naturally occurring phytochemicals such as flavonoids, polyphenols, and other nutrients play a crucial role in maintaining normal health as well as in different pathological processes (Penta *et al.*, 2017). Fruits, vegetables, seeds, flowers, leaves, and bark represent huge reservoirs of phytochemicals such as polyphenols, flavonoids, isoflavonoids, proanthocyanidins, phytoalexins, anthocyanidins, and carotenoids (Singh *et al.*, 2014). Tea (Camellia sinensis; Theaceae) has been consumed as a popular beverage worldwide and skin photoprotection by green tea polyphenols (GTPs) have been widely investigated (Singh *et al.*,2014).

Several natural bioactive phytochemicals have been shown to exhibit epigenetic modulatory capability and act as chemo-preventive as well as therapeutic agents (Penta *et al.*, 2017). Likewise, these natural agents in combination with sunscreens or skin care cosmetics may offer a rational approach for reducing skin cancers and other skin diseases (Singh *et al.*, 2014).

**Treatment**

Not always success has been achieved. Most patients appear for medical help in very advanced stages of the disease. This and the wrong treatment option are the two main causes of failure.However, in case of advanced stages of cancer, surgery will not help in controlling the deadly disease, wherein the cells get metastasized into distinct body organs such as lymph, lung, and liver

(Penta *et al.*,2017). In the process of tumour progression, one of the primary functions of the blood and lymphatic vascular networks is to help tumour cells escape immune surveillance (Wang *et al.,* 2017).

Retinoids influence epidermal differentiation and are used to treat and prevent skin cancer, therefore, with further investigation, TIG-3 may become the first molecular marker of aggressiveness and of retinoid action during treatment or chemoprevention studies (Duvic*et al.*,2000). They have been studied extensively for their potential as therapeutic and chemo-preventive agents for a variety of cancers, including nonmelanoma skin cancer (NMSC) (Cheepala*et al.*,2009). The current chemotherapeutic agents for the treatment of skin cancer are associated with several adverse effects (Penta *et al.*, 2017). Anecdotal evidence suggests that non-surgical treatment has increased since 2002 for superficial BCC with imiquimod in particular (Doran *et al.*, 2015). Recently, physicians have been empirically combining two treatment approaches, topical 5-fluorouracil (5FU) and photodynamic therapy (PDT), but without any scientific basis (Maytin*et al.*, 2018).

Most of the alternative treatments used in cancer therapy fall into one of the following categories: biologic and pharmacologic therapies, immunologic therapies, herbal therapies, metabolic therapies, mind-body therapies, and nutritional therapies (Balch and Balch 2000). Alternate the following in your cancer prevention or cancer therapy program: astragalus, birch, burdock root, cat's claw, chaparral, chuchuhuasi (a rainforest herb), cranberry, dandelion, echinacea, fennel, green tea, licorice, macela, milk thistle, parsley, paud'arco, red clover and suma (Balch and Balch 2000). The use of herbs for medicinal purposes has increased dramatically over the past decade (Tierney *et al*,2003). Agents that are commonly used include green tea, echinacea, essiac tea, flaxseed, mistletoe, and coenzyme Q, as well as others (Tierney *et al.*,2003).

Surgery is the prime treatment for NMSC: more than 70 % of the BCC lesions recorded in the 2002 National Survey were surgically excised (Doran *et al.*, 2015). Direct costs accounted for 72% of costs ($10,230 per melanoma and $2336 per non-melanoma) and indirect costs accounted for 28% of costs ($34,567 per melanoma and $123 per non-melanoma) (Doran *et al.*, 2015). Direct costs include the management of skin cancer from diagnosis, follow-up treatmentand refer to the utilization of health care resources such as hospital, medical and allied health care services, while indirect costs reflect the lost productivity resulting from an individual’s inability to work (morbidity costs such as sick leave and early retirement) and premature mortality (defined as death before the age of 65years, the upper limit of the working age in Australia) (Doran *et al.*,2015).

The local treatment with NILS including the diagnosis, treatment and follow-up, starts at 200 Euro for NMSC and 1000 Euro for Melanoma, while for large size lesions the cost dependsonthe location, ulcerating form etc.

Complementary and alternative medicine have played an increasing role in the treatment of many diseases, including skin cancer. The therapeutic uses of garlic in cancer have been widely studied, as well as several other phytochemicals also have been reported for their imperative effects against skin cancer (Singh *et al.*, 2014). Many phytochemicals such as sulforaphane, tea catechins/epicatechins, curcumin, and resveratrol have been shown to have antimelanoma activity (Penta *et al.*, 2017).

The use of NILS in both early and advanced stages of the disease proved to be effective in cases of BCC, SCC and MM. Therefore, in cases when the aforementioned treatment methods are ineffective, contraindicated in the patients' health circumstances, patients suffer from Xeroderma Pigmentosum disease or Basal Cell Nevus Syndrome for which care is required time after time, refuse to receive standard medical treatment and have lack of systemic side effects and toxicity, using NILS would be advisable.

## 5. CONCLUSSIONS

The beginning signs of skin cancer involve a change in the skin. This may mean that a new lump or sore has formed on the skin, that a new mole has popped up, or that an existing mole has begun to grow or change in shape. There might be devastating consequences of missed diagnosis and the delayed treatment of malignancy or the unnecessary treatment of lesions. The ALMS "STROKA" Clinic in Tirana, Albania has a long history in the realm of alternative treatment in patients with malignant melanoma and non-melanoma skin cancer (NMSC). This treatment method is tailor-made for all the patients and in particular for the inoperable patients, patients that underwent surgery, radiotherapy, etc. Alternative treatment is applied to patients when the skin tumor has not affected the nearby lymph glands, bones and various organs. In 7 cases cancer was found in an aggressive form and indomitable from NILS. The patients were diagnosed with BCC and SCC. There are cases with prolonged illness, ulcerated and moisturized tumor. In these cases, the patients underwent corticotherapy or radiotherapy etc. This treatment generally avoids skin transplantation at the tumor site.

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# THE ECONOMIC POLICY AND THE VALUE OF HUMAN CAPITAL STOCK IN ALBANIA

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## ABSTRACT

In the present paper the impact of the economic policy of the Albanian government to the growth of the value of human capital stock in the country is reported through a survey of four key elements of the government's economic policy of the recent years: i) the employment policies, ii) the education policies, iii) health care policies, and iv) state insurance policies. The analysis of the expenditures and investments made in these areas enables to reveal the direct and indirect relationship of these elements to the indicator 'value of the human capital'. The research is based on the classic forms of assessing the impact of the social and health policy to the value of the human capital stock. The study distinguishes the relevant benefiting age groups, focusing particularly on the ages of 7-24 for the impact of education; ages 65-75 for social and health insurance policies and ages 40-67 for employment stimulation policies due to the budget drafted based on the aforementioned age groups. Consequently, efforts have been made to approximate the Albanian statistical calculations according to the J-F protocol algorithm, with the dynamics of these policies in the last six years. "The value of the human capital stock" is one of the most novel indicators that determine the development potentiality of an economy. There are many macroeconomic recommendations that this indicator should be considered as an important standard of a country's national statistics, because it calculates a potential development trend of a generation and of a whole nation over time. Nowadays it has become a prominent indicator of importance in the current assessment of a society, as well as an indicator of its potentiality for development.
***Keywords****:* value of human capital stock, development multiplicator, employment reforms, opening of health market

## 1. Introduction and historical development of the concept

There is a long history in the realm of the economic thought with regard to the human capital. However, its numerical evaluation has been introduced only in the last decades. It became especially important after the 2000s when it was officially classified as an indicator not only of economic development, but of social welfare too.

Organization for Economic Co-operation and Development (2012) defined the human capital to be *entirety of man's individual knowledge, skills, competencies and attributes that facilitate the creation of personal, social and economic well-being* as based on the 2001 treaty.

At a first glance it appears that human capital is a product of individual and genetic features of humans. Nevertheless, all the surveys reveal that in some way it is not only a genetic element but also social production that, like all forms of capital, is accumulated and increased (Schultz 1961). It is this feature that associates this concept firstly with education, employment, social policies of the government for social and health protection. In this aspect, it is one of the key elements related to the government development policies on one hand and market dynamics on the other. Because of the relations with the governmental development policies, its own dynamics expresses the potential of a country's market development as well as the growth of the set of parameters of quality assessment of its citizens' lives.

Over the past 20 years, the economists' efforts to set up various macroeconomic indicators which determine the degree of satisfaction of a country's population have been increasing (Gibson and Oxley 2006; Li 2010; Liu, 2011). There are different theories evolved about this, but everyone recognizes a prominent difference between the value of the stock of human capital and the level of social satisfaction of a country's population. If the first concept is represented by a number and there is a certain protocol for measuring it, there are many attempts to calculate the second one, but there is still a lack of a measurement protocol approved by the relevant institutions and an international comparison base.

The concept of social happiness is widely treated in social psychology as a perception of the satisfaction level of the population in a given country. As declared by Stiglitz (Financial Times, 2009), the French President Nicolas Sarkozy established a commission with eminent economists conducted by Nobel laureate Joseph Stiglitz to develop other economic indices of happiness outside the classic concept of GDP or "Human capital stock value". This commission did take into account a new indicator called GDH (Gross Domestic Happiness) that has been used in the state of Bhutan, in the Himalaya to replace the GDP and that was considered by the Prime Minister Jigmi Y. Thinley as of a greater importance than the economic indicators (The Guardian, 2012).

Gross Domestic Happiness (GDH) is an indicator for which there is no defined assessment protocol, but everyone accepts that it is related to four key elements: i) the ecologically supported sustainable development, ii) the development of education as a function of rising social conscience, iii) the extinction of fear and the re-conception of freedom and, iv) the evaluation of time as a function of perspective.

Among the four aforementioned indicators none of them is of pure economic sense. However, these indices indirectly do assess the civic behavior influenced by external economic elements. The only conclusion the "Sarkozy Commission" could draw was that in the countries where the public administration worked better, the population felt happier. The comparison between the "Human Capital Stock Value" and the GDH (Gross Domestic Happiness) is done not only to figure out new statistical evaluation systems of economic developments, but also to analyze the historical difference of a protocolled indicator with a non-protocolled but evidenced one. The measurement of the indices related to the development potentials and the level of civic satisfaction is a signal of the change in the classical economic assessment, as well as a sign of the expansion of the economic progress concept.

## 2. METHODOLOGY

Human capital stock value is of great importance for the researchers involved in the area when analysing factors and development opportunities. World Bank for the first time made evident in 2006 these four methods to calculate the value of human capital: i) calculation based on the cost of education (cost-based), ii) calculation based on the ability to generate income (income-based), iii) calculation based on the effectiveness of the education process (all levels) and, iv) calculation based on the cost of raising and educating children aged 0-26 (WB 2007; Nosvelli 2009).

After 2008, many countries have engaged special public units to calculate the value of human capital and all accepted the so-called Jorgenson-Fraumeni protocol (known as the JF method), which is an analytical calculation of the incomes generated by the individual for all working years at full value (full labor compensation) (Jorgenson and Fraumeni 1992 a;b). A better understanding of this methodology can be achieved considering these key elements: i) to calculate a person's life cycle in the methodological aspect of the study, in countries where the retirement age is determined by law, the population is classified as follows (as the value of human capital stock): a) age group 0-4 years old, where there is neither education nor employment, b) age group from 4 to 15 years old, where it’s education only, c) age group from 16 to 40, where it is education and work, d) age group 40-67, where it’s only work and, e) age group over 68, where it’s only retirement.

These age groups are classified as the life span corresponding to working age and the time of life outside the labor market. Since the first and second groups are out of the labor market/ working age and do not generate incomes, the calculation of the value of human capital stock is impossible with the income method. Consequently, it is generally accepted that direct calculation of capital stock value should consider the population of group 16-67 years old and over 68, for the incomes from pensions, ii) to approximate the calculations in respect to the methodological use of the formula, it is more correct to divide the age group 16-40 from the 40-67, because for the first one there might be education and job as well, while for the second age group there is only job during that part of the lifespan. This means that we have two different algorithms for these age groups and two different scores in terms of value, iii) all algorithms used in the estimation of the stock of human capital belong to the descending system from the highest level. For instance, for a 75-years old person (average life span), the income of the 75'th year of life in the calendar year 2017 is A, while those for 2018 (next year) is equal to zero. For a 74 years old income in 2017 is A, while in 2016 it is B. For a 73 years old income for the next two years are A + B and C for the year 2015. Following this system all the groups with the same level of education, but different ages have an individual value with the real income of the current year and a future income value equal to the oldest people in the same education group. This formula is valid to measure the value of the human capital stock in 2017 for all persons in a country that in that year range from 0 to 75-years old, where the value of the 75-years old stock is added to the one of the 74 years old and so on.

This is presented in the following formula:

$$LIN_{age}^{edu} = EMR_{age}^{edu} * AIN_{age}^{edu} + SUR_{age+1} + LIN_{age+1}^{edu} \{(1+r)/(1+\delta)\}$$

where:

$LIN_{age}^{edu}$ - is the present value of income from work, for individuals with education level (edu), at age(age) as the amount of expected income realized in the working life cycle;

$EMR_{age}^{edu}$ - is the level of employment of persons with education level (edu) at age (age);

$AIN_{age}^{edu}$ - is the actual income (as income statement) for individuals with education level (edu) at age (age);

$SUR_{age+1}$- is the probability to live a year longer (age + 1);

$LIN_{age+1}^{edu}$ - is the present value of income from work for an individual with the same level of education but one year older;

r- is the forecast of the annual growth rate of future labor income (in real terms) for a person with these characteristics

δ- is the forecast of annual inflation rate in the future (in real terms).

The protocol for measuring the value of the human capital stock considers not only the labor income (generated during working time), but also the income generated by the productive exploitation of hours beyond work time (off-labor income). The methodology in this case is even more complicated and is calculated using the relationship of the two elements wage / revenue in off-labor activities. The methods mostly used for this calculation, according to economic literature, are: i) opportunity cost method (indirectly measurable), ii) Replacement Cost Method (comparatively measurable).

*The opportunity cost method* relies on the principle of assessing the added value of working time (which determines market income) that is conditioned by use of non-working time. For instance, physical exercise (non-work time) potentially influence working time with an increase in productivity. Hence, even though no direct income is generated for this time-period, additional income is generated at the working time. Theoretically, health welfare (as the value of rest time) at worst is equal to the value of the work productivity increase. In fact, this method is nothing more than a way of finding an indirect equivalent assessment for an element that can't be directly assessed. Consequently, it bears all the error' elements that contain the statistical methods of calculation based on comparative evaluation methods (Jorgenson and Fraumeni 1992 a;b).

*The replacement cost method* relies on another valuation, namely the assessment of the outcomes from off-labor market activities by the possible cost of its realization from a market subject that provides this service. The hours a person uses for house chores can be estimated by the value of a housemaid, or the value of the working time a person spends to make a hydraulic adjustment at home is equivalent to the cost of paying a plumber for this job (Liu 2011; d'Ercole 2012).

These methods both require statistical data relating to the use of leisure time, which might be provided by complex surveys based on age, education level, gender and other characteristics of the population.

Using the above-mentioned protocols and many highly-polled surveys and studies in this regard, many developed countries have determined the value of the human capital stock over a given period of time. It is a common use that the key factors determining the level of human capital value of a country are: i) educational level and professional qualification of the population realized at certain milestones of the working cycle, ii) the level of health care and life quality indicators of a country's population, iii) the level of economic development and its standard evaluation indicators, iv) the population structure, its average age and the probability of extending the life cycle and, v) social and employment policies and the creation of facilities for the initiation and consolidation of new businesses.

The above discussion aims at presenting the methodology for human capital measuring under the Jorgenson and Fraumeni protocol and factual forms of calculating this indicator from the specialized institutions in developed countries. Albania is missing this experience. INSTAT (Institute of Statistics) has not yet taken real steps to calculate the value of human capital stock, consequently any effort to make such calculation does not comply with any relevant protocol.

## 3. RESULTS AND DISCUSSIONS

Develop of an alternative indicator of human capital stock value in Albania has not yet been calculated and the implementation of the measurement protocol of this indicator has many difficulties, not only related to the application of measuring algorithms but also to the definition of the data which can be included. The inability to complete all the data of the J & F formula for estimating the stock of human capital, has encouraged us to the development of an indicator that comes closer to the value of human capital stock, but it is not rigorously calculated with the protocol of this indicator as above described. The attempts are done to compile an indicator that could reflect how much income an Albanian individual could generate throughout his/her life.

*The first question* raised related to the concept of the value of human capital stock is: "How much income a 29-year-old average citizen in 2017 will earn for the next 38 years until he retires?" We have chosen the 29-year-old because in the statistical classification of labor force participation defined by INSTAT the population is classified in two groups: 15-29 years old and 30-67 years old (INSTAT 2018). The first group consists of persons who have a job and study, whereas in the second group people are mainly only working. On the other hand, according to the existing laws in 2017, the retirement age in Albania is 67.

*The second question* is: "How much income do Albanians aged 16-29 years generate?" The method of calculating the potential earnings that this group generates is related to the age group methodologies that study and work at the same time. The analysis below has used data from INSTAT related to students enrolled in public and private secondary education schools, and higher education at the first and second levels of study. Participation in the workforce of this group is already analytically determined in the relevant labor force survey calculated by INSTAT.

*The third question* is: "How much income do Albanians make by the time they retire to the age of 75?" In answering these questions, we have taken into account the average pension any Albanian retired in 2017 gets, and the amount received in the same year by those who get pension in their 75-th year of life.

*The fourth question* is: "Is it possible to determine the value generated by Albanians aged 0-16 years based on the social costs of raising this age-group?".

Our estimation of the indicator that indirectly assesses the potential of the Albanian population to generate income during its life will be supported by the following judgement and argumentation as for the 16-67 years old group the degree of participation in the workforce of the Albanian population as per the INSTAT classification for this age group, is reported through a two-step division, 16-29 and 30-67 years old. The participation of the first age-group in the labor force from 2015 to the end of 2018 is on average 46.5%, while for the second age-group it is on average 76.5% for the same period. The Labor Force Surveys, Tr.1.2015-Tr.4.2018 published by INSTAT, stated that the participation in the working force for the population group 15-67 is on average 66.4% (INSTAT 2018). Based on these data, and estimating the fact that in the classification of our system for both groups we do not have analytical data on the average salary level for each group, we have made all the calculations by mean of an average level salary. Since the participation level/ percentage in the labor force for these groups is different, the value of the labor income for the demographic range of these groups will be different. Specifically, for the age group 16-29, only 5 out of 10 people work and the income of 5 persons as an estimate of the group`s earnings is distributed to 10 persons, whereas for the age group of 29-67, there are 8 out of 10 people that work, and their income is distributed to 10. Therefore, even if the average salary is equal between both groups, the income value generated by each group is different. In this respect, we have partial compliance with the Jorgenson and Fraumeni algorithm, even though this compliance is not based on the figures, but on the methodology.

i. Since the basis of our calculation is a 29-year-old citizen in 2017, the indicator that will be derived from this study is a value that belongs to this year. In this respect, the potential earnings of this 29-year-old citizen will be calculated until retirement based on the average salary of this year, the average annual pension of that year and the average longevity of Albanians in the same year. Thus, starting from the average salary of 2017, we should calculate the income from the work of the 29 years old up to 2050, the income of the pensioners up to 2025, the budget expenditures for kindergartens from 2012 to 2017 and the budget expenditures for education and health in 2005-2017.

ii. The calculations are based on the number of Albanian people living in the country, as determined by INSTAT in 2017. The time value of money is considered in order to calculate the income that can be provided with the average salary of a 29-years old by year 2050. It has been processed with the macroeconomic parameters defined below.

iii. The underpinning to our calculations were some data for the next 30 years that are important for the progress of the country's economy, more specifically: a) the average annual growth of the Albanian economy for the next 30 years is 1.3%. (INSTAT, 2018), b) the average rate of inflation for the next 30 years is 2.8%. (Celiku *et al*., 2010), c) long-term treasury bill interest for the next 30 years is 6.3%. (Cani and Hadëri 2002). These forecasted indicators are provided from studies made by the governmental entities or prominent Albanian specialists and they have derived from well-known statistical methods.

iv. In order to compile a statistical indicator comparable to the concept of the value of human capital stock by age groups, we have calculated the income that Albanians can generate in 2017 as follows: a) based on the average salary of 2017, according to the time value method we have calculated the probable income of a 29 years old until his/her retirement age that by law was 67, b) based on the average pension level in 2017, according to the time value method we have calculated the probable income of a 67 years old until the 75th year of his/her retirement, c) after correcting the average salary according to the participation in the working forces of the age group 16-29, we have calculated the expected income of this age group and added this value to the budget and private expenditures for the high and higher education for the years 2004-2017 divided by number of pupils and students, d) for the age group 0-5 years we have used the comparative cost method according to the level of budgets for private and state pre-school education for the years 2012-2017, e) for the age of 6-16 we have calculated the incomes according to the comparative cost estimates of primary education (9 years of education) expenditure for the years 2012-2017 divided by the number of pupils.

Based on the aforementioned data, the indicator for "human capital stock value" in Albania for 2017 is approximately 16,250,000 Lekë (ALL). This is a totally personal assessment that will certainly change with the perfection of the measuring systems of this concept. We do not pretend to provide the accurate value of human capital stock in Albania, but to approximate a figure that can create the idea of the value of this indicator. In this respect, the number resulting from our estimates falls into an interval of plus - minus 10%, which is affected by the potential change of the aforementioned factors and the probability of their impact.

## 4. CONCLUSIONS AND STUDY LIMITATIONS

The present paper aims to address an indicator that serves "the value of the stock of human capital in Albania". The main elements that limit the study methodology and its result among others are: i) the age groups defined by our calculating system could not be fully matched with those of the J & F algorithm, but the calculating character is incorporated. It is true that the income tax calculations have not considered the inequalities of income that come from the educational level. It was almost impossible to add this factor to our calculations. Until today in Albania, there are no analytical data which correlate the degree of education to wages. We accept that this calculation has an error degree, which is higher for the group of 16-29 years old, whereas for the group over the age of 50 is much less sensitive. Once work experience becomes an important element of the work value, the impact of the education factor is far less significant, ii) taking the average salaries as the basis of income from work, we have actually seen the direct impact of age on wages, but on an average level. This does not mean that the same person named A.B. who was employed in 2001 in the private sector, earned a wage of 13,355 Lekë (ALL) and in 2018 the same employee had a monthly salary of 50,589 Lekë (ALL). These are data on the average wages and hide the "average error". In this respect, we are biased from the J & F system, where the income of a 67-years old with x education having y income, means it in real terms in 2017. In our system, the income of the 67-years old is calculated as the expected wage of the 29 years old in 2050, iii) if we have a figure that determines the expected value created as income from Albanians for the four age groups, then we can say that we have compiled an indicator that can approximate the economic value of the human capital stock in Albania. Surely, we are dealing with a complicated concept of the time value of money, which is conditioned by the macroeconomic dynamics of the country and several other macroeconomic factors. All these factors will affect the result in different ways and magnitudes and will condition the variation of the value in an interval. Based on a forecast, the real value of the output is more predicted than estimated and, iv) the main difference of our indicator from the value of the human capital stock calculated by other institutions is that this calculation is outside the established protocols and tries to create data rather than administer them. This is the difference between calculation and estimation, where the first administers and delivers results, while the second creates the data and tries to prove their validity.

The employment policy has been one of the main factors that has affected the value of human capital in Albania. From 2014 until the end of 2018, there is a gradual increase in the level of employment, but the most positive elements appeared at the end of 2017, when for the first time in the last 20 years the employment growth was higher than the increase of economic growth (Survey of the work force, INSTAT, 2018). On this basis, we can say that the value of human capital stock for the age group up to 45, which is very vulnerable to employment, has risen more than this figure. The main employment factors that influenced the value of the human capital stock are as follows: i) growth of employment in agriculture in the age group 45-55. Agricultural sector development was extensive but also intensive, especially in some products where export growth focused on agricultural capital and investment in production tools. The development of agriculture, as it occupied most of the workforce, curtailed the phenomenon of internal migration and alleviated social problems in the village, ii) boost to the formalization of the economy. Changes in the tax system by business structures not only restricted informality in the Albanian business, but also limited the possibility of informality in the labor market. In the last two reports of the Labor Inspectorate, for 2016 and 2017, there is a significant reduction in informality that directly has increased the estimated income of the population (Labor Inspectorate, 2017; 2018), iii) compiling policies that stimulate the growth of innovative ventures with Guarantee Funds for all those individuals or businesses that sought to enlarge their business. This policy had the main purpose to stimulate mostly the business activity in the field of production with the aim of enabling systematic industrialization of the country and internationalization of Albanian businesses. A faster growth of medium and large businesses in the last two years has undoubtedly brought about a positive pressure on consumer growth as well.

In conclusion, we would like to clarify here that the "value of human capital stock" in Albania does not belong to someone in particular, it has no name or surname, but it belongs to the Albanians in general, which we do not divide in rich and poor people, neither in well-educated and non-educated ones, but in happy and unhappy ones.

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