Andean Blueberry (*Vaccinium meridionale* Swartz) Seed Storage Behaviour Characterization Under Low Temperature Conservation

Categorización del Comportamiento de las Semillas de Mortiño (*Vaccinium meridionale* Swartz) en Almacenamiento a Baja Temperatura

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Abstract. *A study was conducted to categorize harvest stage, maximun dry weigth and tolerance to desiccation at low temperature storage of seeds of the promising species "andean blueberry", V**accinium meridionale** Swartz. The aim was to determine the long term conservation possibility of a duplicate of the current Colombian Andean Highland field collection in cool storage rooms, as well as that of newly collected local populations from the mentioned highland region, which are in danger of genetic erosion due to human intervention and the ongoing global climate change. Both maximum dry weight and the highest germination percentages were observed to be associated in seeds extracted from deep purple (fully ripe) berries. The seeds exhibited orthodox storage behavior, which indicates the possibility for long-term cold storage of a duplicate of the current metapopulation of the species in the country.*

Key words: Genetic resources, germplasm banks, seed physiology maturity, biodiversity conservation.

Resumen*. Se realizó un estudio para categorizar el estado de cosecha, máximo peso seco de la semilla y la tolerancia de ésta a la desecación y almacenamiento a bajas temperaturas, en la especie promisoria mortiño,* ***Vaccinium meridionale*** *Swartz. El objetivo fue establecer el potencial de conservar un duplicado de la colección de campo y rescatar y almacenar poblaciones espontáneas de la zona alto andina colombiana, en peligro de pérdida por intervención humana y el cambio climático, que pueden causar erosión de la diversidad genética de la especie. El máximo peso seco se logró a partir de semillas extraídas de bayas de color morado oscuro, con obtención de germinación superior en éstas y una relación directa entre el peso seco y el porcentaje de germinación. La semilla exhibió comportamiento ortodoxo, lo cual indica la posibilidad de almacenamiento a largo plazo de un duplicado de la metapoblación de la especie, existente en el país.*

Palabras clave: Recursos genéticos, bancos de germoplasma, fisiología de semillas, conservación de biodiversidad.

Andean blueberry (*Vaccinium meridionale* Swartz) – mortiño, agraz, vichachá or camueza being its Spanish names – is a promising fruit of the family Ericaceae growing in the Andean highland forests of Colombia (Luteyn, 2002). The national demand for this product is currently growing (Corantioquia, 2003) due to its anti-oxidant properties and similar anthocyanin and phenol contents to those of the *Vaccinium* species from other latitudes, as reported in several works (Gaviria *et al*., 2009a, 2009b). These features are particularly true of those *Vaccinium* known as blueberries (Heinonen, 2002), which include *V. meridionale*. The presence of these secondary metabolites has been associated to cancer risk reduction through the inhibition of malignant cells (Matchett *et al*., 2005), to slower progress rates, in both, the Alzheimer disease and premature ageing processes (Joseph *et al*., 1999), to the reduction of cholesterol levels and urinary tract diseases, and to lower cardiovascular disorder risks (Halliwell *et al*., 1995).

The offer of this species in Colombia comes mainly from extractive processes, which in some cases are harmful and result in berries of heterogeneous quality in different ripening states. This, coupled to the growing demand for the product, has led to the establishment of some plantations. The harvesting of wild populations has been reported in different parts of the world, reaching up to 40 and 35 million yearly kg in Finland and Denmark, respectively (see Saastamoinen, 2000 for several references).

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The development of this species, as an economic alternative, requires productive and well adapted cultivars, bearing the quality expected by the market. Along these lines, Lobo (2006), points out that the cultivation of this crop can be based on wild populations from which the most prominent individuals could be cloned. Such a process requires collecting and preserving the available variability of the species and, characterizing and evaluating it by different procedures.

At this point, it is worthwhile mentioning that the wild Colombian populations of *V. meridionale* are currently endangered due to the conversion of spontaneous vegetation areas to agricultural zones, the establishment of illegal crops, the fragmentation of the forests and an unsustainable extraction of the berries.

As a response to this situation, spontaneous populations were located by CORPOICA in the Andean highlands of the departments of Antioquia and Santander, and by Universidad Nacional de Colombia, Sede Bogotá, in the departments of Boyacá, Cundinamarca and Nariño. This allowed establishing a field collection at La Selva Research Center, which belongs to CORPOICA, located in Rionegro, Antioquia; and in Marengo farm, which is located in Mosquera, Cundinamarca, and belongs to Universidad Nacional de Colombia. This was done in order to preserve this germplasm and study its variability and potential use, in productive processes and in the repopulation of forest areas (Medina *et al*., 2009).

Ex situ conservation, of this species, can be accomplished through field and/or seed collections. The first option is expensive, as far as, for security reasons (mostly adaptation problems), it is necessary to keep *in vitro* or *in vivo* germplasm duplicates in other localities. In this respect, Ligarreto *et al*. (2011), mention that the Colombian natural populations of *V. meridionale* are located in, and adapted to, very heterogeneous climate and micro-climate conditions, which implies that some of them will probably not settle when planted in one single *ex situ* locality.

An alternative to field collections are seed banks, which allow the preservation of different natural populations and recombinant clones. In this respect, blueberry self-fertile and self-sterile flowers have been reported by Tanner (1982), and Griffin and Blazich (2008; cited by Shahram, 2007); whereas the European bee has been observed to be an important cross-pollination agent for the species (Brewer *et al*., 1969). It is worth noting that *V. meridionale* is a clonal plant (Albert *et al*., 2005), and thus propagates spontaneously both sexually and vegetatively; in the latter case, through stolons or rizomes (Wilbur and Luteyn, 2008). This determines natural populations to be made up of several clones (Medina, 2010), which supports the notion that seed collection is likely to capture their recombinants. In studying the MDH isozyme locus, Medina (2010) has reported heterozygosis in 36 individuals (out of 40 expected ones under equilibrium conditions) from a sample of 250 Andean blueberry plants collected in three Colombian populations. This is indicative of allogamy (thus implying great odds of obtaining recombinants through seed collection) and, consequently, makes seed conservation a feasible practice in this species, as far as they are found to be tolerant to desiccation.

The preservation of blueberry populations, through seeds, implies knowing if they can be refrigerated for long or medium periods of time (respectively corresponding to orthodox and intermediate seeds), or if they do not tolerate desiccation (recalcitrant seeds). In this sense, Hong and Ellis (1996) have stated that the goal of germplasm banks should be to keep seeds for periods of 10 to 100 years or more.

According to a protocol developed by these authors (ibid.), orthodox seeds tolerate up to 5% moisture levels at low temperatures, while intermediate seeds bear up to 10-12% moisture under cold temperature conditions; and recalcitrant seeds cannot be dried to those levels, which certainly limits their refrigeration. Complementarily, cold storage requires knowing when the seeds have reached their maximum dry weight, which corresponds to the utmost vigor and viability of sexual propagation structures and physiological maturity (PM), an attribute that is determinant for seed quality and storage possibilities (Kathun *et al*., 2009) and, being genotypic in nature, is also influenced by environmental conditions (Kole and Gupta, 1982; Mahesha *et al*., 2001).

Our literature review found no works on the tolerance of blueberry seeds to desiccation and, their consequent long term cold storage possibilities. However, Ellis *et al*. (1985) have found that the Ericaceae, to which the genus *Vaccinium* belongs, exhibit an orthodox behavior, but no specific reference is made to *V. meridionale*. In turn, Hill and

Vander Kloet (2005), observed longer than 20 year viability times in 5 out of 28 *Vaccinium* species whose seeds had been buried in the soil; whereas Haywood (1994), determined that the seeds of *V. arboreum* germinated after four year soil burial. These results indicate an orthodox behavior of these seeds, in as much as they need to keep low moisture levels to avoid germination. Finally, no literature reference was found on the PM of *V. meridionale* seeds.

On these grounds, we have conducted the current study whose aim was to establish the tolerance of *V. meridionale* seeds to desiccation and, consequently, the long term possibility to store duplicates of the field collection through physiologically mature sexual propagation units.

MATERIALS AND METHODS

Location. The study was carried out at the Seed Laboratory of the Germplasm Bank for Food and Agriculture of the Colombian Nation, located in La Selva Research Center (CORPOICA, Rionegro, Antioquia, Colombia).

Biological material and seed extraction. The seeds were obtained from berries of *V. meridionale* collected at different developmental stages, with the aim of determining their tolerance to desiccation and maximum dry weight, which are indicative of the storage mode they require. The fruits were collected from a natural population at Arví Park, locality of Santa Elena in Medellin, Antioquia (2,500 masl, average temperature of 14.9 °C and yearly average precipitation of 1,998 mm), which corresponds to a very humid, low montane forest (Zuluaga, 2005; Espinal, 1977). Seed extraction was carried out by placing the berries in water and, manually separating them from the flesh. The macerate was placed on a sieve, which received pressurized water, being the seeds retained on the sieve. Then, they were dried on absorbent paper napkins at 17 °C and 78% relative humidity for a period of 48 h, according to methodology by Medina *et al*. (2008).

Maximum seed dry weight. The harvesting stage at which PM is obtained, which also corresponds to maximum dry weight (Kathun *et al*., 2009), was assessed through berry color change during ripening. This allowed defining four sexual propagation unit stages according to dry matter increase (Figure 1):

Stage I: Reddish color covering 25 to 50% of the fruit surface
Stage II: Red color tone covering 50 to 75% of the fruit surface
Stage III: Distinct red on 75 to 100% of the fruit surface
Stage IV: 100% purple color of the fruit surface

After classifying the fruits in these categories, their dry weight was determined in an Ohaus-MB200® moisture meter, where they were kept until reaching constant mass. For such purpose, four repetitions, of 200 seeds each, were measured at each color category.

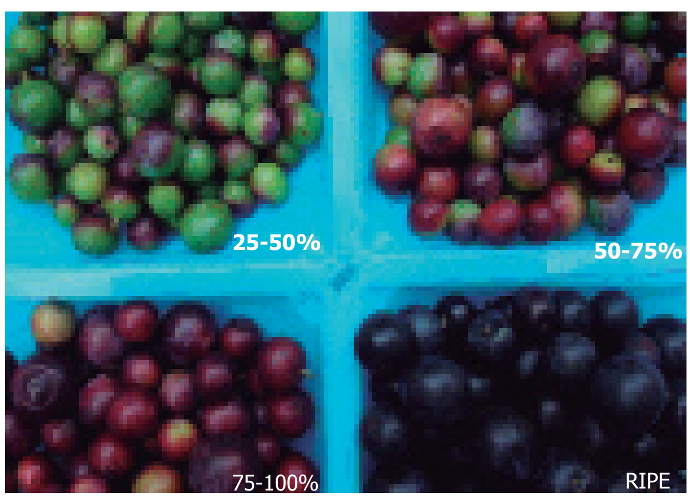

Figure 1. Developmental stages of Andean blueberry fruits (*Vaccinium merdionale* Swartz.) as assessed through external color, in order to evaluate seed dry weight.

Germination tests were carried out in germination chambers at 20 °C. As blueberry seeds have shown positive photoblastic (i.e., light stimulated) germination (Hernández, 2008; Hernández *et al*., 2009), red light promoting germination percentages of up to 90% in *Vaccinium* spp. seeds (Giba *et al*., 1995); the experiment was carried out under alternating 12:12 h light and darkness periods making use of 20 W, 110-120 V/50-60 Hz lamps. The experimental unit contained 100 seeds, with four repetitions placed in Petri dishes with periodically moistened filter paper during the 30 days of the test.

Physiological behavior during storage. The protocol of Hong and Ellis (1996), which allows determining seed tolerance to desiccation, conservation possibilities, and adequate temperature conditions, was applied to find the optimal storage conditions for the studied seeds, which were obtained from category IV fruits, for their drying process. They were included in hermetically closed Fisher Scientific® chambers and mixed with silica gel in equal proportions. The chambers were placed in a room with moisture free circulating air (14% relative humidity) at 29 °C, for the seeds not absorb water during weighting. Initially containing 22% moisture, the seeds took 12 h and 15 min to reach 10% moisture, 19 h and 45 min to attain 7.5%, and 29 h to get to 5%, which are the usual moisture values employed to characterize tolerance to desiccation and corresponding storage conditions.

Viability loss was assessed through germination tests conducted on category IV seeds, which exhibited the highest dry weight. The tests consisted in three temperatures (10, 0, and -10 °C), three seed moisture percentages (5, 7.5 and 10%) and three storage times (4, 8 and 12 months), plus a control corresponding to recently extracted seeds. Germination percentages as obtained after 30 days of the experiment were transformed to arcsine, as usually recommended for this type of variable (Gómez, 1997), and subjected to analysis of variance in SAS 9.0 (Statistical Analysis System). For such purpose, a 3X3X3 completely randomized design with four repetitions was applied. The factors corresponded to storage temperature, seed moisture content, conservation time, and an additional treatment: initial seed germination, which was used as control.

Experimental hypothesis. Based on reported performance of stored *Vaccinium* seeds (Ranwala and Naylor, 2006), the null hypothesis stated that the seeds of ***V. meridionale*** exhibit an orthodox behavior, thus allowing the long term preservation of maximum dry weight propagation units, under conditions of low temperature and moisture content.

RESULTS AND DISCUSSION

Maximum seed dry weight. Figure 2 shows the progression of germination percentage and dry weight of 200 seeds, as functions of fruit color (ripening).

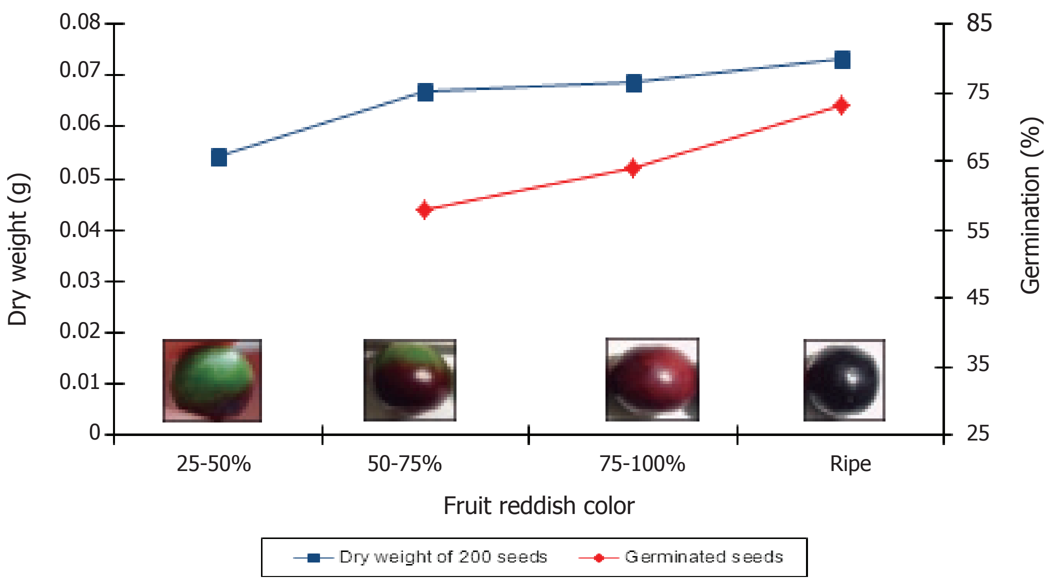

Figure 2. Progression of dry weight and germination of 200 seeds of Andean blueberry (*Vaccinium meridionale* Swartz) during four fruit ripening stages.

As it can be observed, the two former parameters increase steadily with ripening, which starts from 25 to 50% reddish epidermis (stage I) and ends up when it is 100% purple (stage IV). The relation between dry weight and embryo emergence was explained by the model Y = 2,238X – 88.276, R^2 = 0.72; where Y is germination percentage and X is weight of 200 seeds, both parameters reaching their highest scores at stage IV, which is therefore, and by definition, considered to correspond to PM (Kathum *et al*, 2009).

The matching fruit external color as a criterion to judge PM has been mentioned by several works conducted both abroad (Harrington, 1972; Petrov *et al*, 1981; Demir, 1994; Demir and Samit, 2001; Oladiran and Kortse, 2002 and Shivashankaragouda *et al*., 2007, among others) and in Colombia. Thus, Lobo *et al*. (1984) achieved the conclusion that PM in *Solanum lycopersicum*, sin. *Lycopersicon esculentum* Mill was reached at maximum carotenoid pigment content, corresponding to an intense red color on 100% of the surface of the fruit. Just as well, Cárdenas *et al*. (2004), found PM at 100% pericarpium coloration in tree tomato (*Solanum betaceum* sin. *Cyphomandra betacea* (Cav.) Sendt) and at 25% in lulo (*Solanum quitoense* Lam.).

The matching between maximum dry weight and PM has been reported by Copeland and McDonald (2001), who state that before that point seeds are less viable and have a shorter shelf life. These authors state that blueberry seeds must be obtained from fully ripe (purple) berries for better viability and longer preservation potential.

Table 1 shows the performance of seeds obtained from stage IV (fully ripe) fruits germinated (i) immediately

Table 1. Germination of blueberry seeds (*Vaccinium meridionale* Swartz) stored with different moisture contents at -10, 0 and 10 °C.

Storage (Months)	Temperature (ºC)	Moisture (%)	Germination (%)**
0*	20	20.0	76
4	10	5.0	61
4	10	7.5	65
4	10	10.0	62
4	0	5.0	64
4	0	7.5	60
4	0	10.0	60
4	-10	5.0	58
4	-10	7.5	53
4	-10	10.0	60
8	10	5.0	57
8	10	7.5	53
8	10	10.0	55
8	0	5.0	48
8	0	7.5	60
8	0	10.0	48
8	-10	5.0	53
8	-10	7.5	54
8	-10	10.0	51
12	10	5.0	73
12	10	7.5	72
12	10	10.0	72
12	0	5.0	64
12	0	7.5	67
12	0	10.0	64
12	-10	5.0	74
12	-10	7.5	72
12	-10	10.0	60

* Control treatment consisting in recently extracted (unstored) seeds.
** No significant differences were observed between germination averages as they resulted from the arcsine angular transformation.

after collection at 20% moisture content (control treatment) and (ii) after 4, 8 and 12 months of storage under three contrasting temperatures (-10, 0 and 10 °C) and, with three different moisture contents (5, 7.5 and 10%). The analysis of variance did not reveal significant embryo emergence effects along the experimental time resulting from storage temperature or seed moisture content; nor from the interactions between temperature X storage time, moisture content X storage time, and storage temperature X moisture content X storage time. The only significant differences were observed between all pooled data average records (including the control treatment) along experimental time. The non-significant effects of seed moisture content or storage time and temperature on the germination of the studied *V. meridionale* seeds, indicates that their behavior is orthodox, i.e., they tolerate storage at low temperatures with 5% seed moisture contents, which are the conditions usually recommended for long term storage. Also, noteworthy is the fact that, along the 12 months covered by the trial, the low temperature treatments did not affect germination even at 7.5 and 10.0% seed moisture contents (which are likely to determine viability losses), all of which corroborates the orthodox behavior of these seeds.

The current results support the notion that the seeds of the Ericaceae are orthodox (Ellis *et al*., 1985). This has been specifically demonstrated for several *Vaccinium* species, among which we can count a series of temperate zone blueberries whose survival, under cold storage conditions, was studied by Darrow and Scott (1954) during a period of 12 years. It is worthwhile noting here that the tropical Andean highland blueberry (V. meridionale) belongs to this clade. Just as well, Ranwala and Naylor (2006) observed the orthodox behavior of bilberry seeds (*V. myrtilus*), and Barney *et al*. (2009) reported the longevity of huckleberry (*V. cespitosum*) seeds stored at cold temperatures during 30 months. In turn, Shafii and Barney (2001) have documented the effectiveness of storing black huckleberry (*V. membranaceum*) low moisture seeds at cold temperature for a period of seven years.

The current results are positive for the development of this species as a productive alternative, as far as they support the long term conservation of germplasm collected from populations of interest. This is important to prevent the loss of the necessary variability for the cropping of the species, all the more when the natural populations are endangered by deforestation resulting from the agricultural conversion of natural areas, which includes the planting of illegal crops (Medina, 2010). In addition, the currently ongoing climate change is likely to take a toll on population resilience, with the consequent genetic erosion of endemic attributes, which is acknowledged as unavoidable in the short and medium terms, and has been recently reported (Andrade *et al*., 2010). Along these lines, Campbell *et al*. (2009), as well as the Convention on Biological Diversity (CBD, 2009), have asserted that adaptation strategies that incorporate natural resources tend to benefit both people and biodiversity.

The fact that blueberry seeds are orthodox in nature facilitates the preservation of previously collected populations, as well as the rescue of additional ones before they get lost, which corresponds to their value of existence. In addition, preserved metapopulations can be planted as field collections in order to characterize their attributes such as variability and potentialities. This confers the material its value of option, which is useful in the selection of high quality individuals or parents with desirable features for hybridization processes, leading to cultivar development, which corresponds to utility value.

In this respect, Jump *et al*. (2009) have pointed out that anthropogenic changes are likely to alter dramatically the pressures acting on natural populations, thus threatening their genetic diversity, which is, in turn, determining for security in agriculture and crop industries and their adaptivity to environmental changes, which corresponds to their value of option. Therefore, the importance of the orthodox behavior here found in the studied blueberry seeds, as far as it facilitates the long term preservation of the natural populations of this species.

CONCLUSIONS

The studied seeds of *Vaccinium meridionale* reached their maximum dry weight at fruit color stage IV (purple). These seeds exhibited higher germination percentage, which indicates the feasibility of their long term conservation.

The seeds of this species were found to present orthodox behavior, in as much as they conform to the storage standards that define this category (-10 °C and 5% moisture content), which was confirmed by application of the protocol of Hong and Ellis (1996). No statistically significant decline of the studied propagation material resulted from storing seeds with contrasting moisture contents (5, 7.5 and 10%) at different temperatures (-10, 0 and 10 °C). Said effect was assessed by comparing treated seed germination with that of the control treatment. Hence, the seeds can be said to tolerate storage under the evaluated desiccation and temperature conditions.

Long term storage at low temperature allows obtaining a security duplicate of the existing *V. meridionale* field collection, thus protecting its value of existence, which is likely to acquire option and (eventually) utility values after the characterization of its variability.

Provided that *V. meridionale* is a promising species with growing demand in Colombia, and that there is still uncollected variability that is endangered by diverse anthropogenic processes and by the ongoing climate change, it is advisable to preserve the unique biological capital that such unsampled populations represent for the country by applying the procedure tested in this work: long term storage at low temperature. This is now a feasible option, due to the orthodox behavior observed in the studied seeds.

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BIBLIOGRAPHY

Albert, T., O. Raspe and A.L. Jacquemart. 2005. Diversity and spatial structure of clones in *Vaccinium uliginosum* populations. Canadian Journal of Botany 83(2): 211-218.

Andrade, A., B. Herrera and R. Cazzolla. 2010. Building resilience to climate change. ecosystem-based adaptation and lessons from the field. IUCN's Ecosystem Management Series No. 9. International Union for Conservation of Nature, Switzerland. 164 p.

Barney, D.L., O.A. Lopez, B. Shafii and W.J. Price. 2009. Effects of stratification and cold storage on seed germination characteristics of dwarf huckleberry. Acta Horticulturae 810:591-598.

Brewer, J.W., R.C. Dobson and J.W. Nelson. 1969. Effects of increased pollinator levels on production of the highbush blueberry. *Vaccinium corymbosum*. Journal of Economic Entomology 62(4): 815-818.

Campbell, A., V. Kapos, J.P. Scharlemann, P.W. Bubb, A. Chenery, L. Coad, B. Dickson, N. Doswald, M.S. Khan, F. Kershaw and M. Rashid. 2009. Review of the literature on the links between biodiversity and climate change: Impacts, adaptation and mitigation. Technical Series No. 42. Secretariat of the Convention on Biological Diversity (CBD), Montreal, Canada. 124 p.

Cárdenas, W., M.L. Zuluaga y M. Lobo. 2004. Latencia en semillas de lulo (*Solanum quitoense* Lam.) y tomate de árbol (*Cyphomandra betacea* (*Solanum betaceum*) Cav. Sendt.). Plant Genetic Resources Newsletter 139: 31-41.

CBD [Convention on Biological Diversity]. 2009. Biodiversity and climate change mitigation and adaptation: Report of the second ad hoc Technical Expert Group on Biodiversity and Climate Change. Technical Series No. 41. Secretariat of the Convention on Biological Diversity. Montreal, Canada. 126 p.

Copeland, L.O. and M.B. McDonald. 2001. Principle of seed science and technology, fourth edition. Kluwer Academic Publishers, Boston. 488 p.

Corantioquia. 2003. Conozcamos y usemos el mortiño. First edition. Corantioquia, Medellín. 24 p.

Darrow, G.M. and D.H. Scott. 1954. Longevity of blueberry seeds in cool storage. Proceedings of the American Society for Horticultural Science 63:271.

Demir, I. 1994. Development of seed quality during seed development in okra. Acta Horticulturae 362: 125-131.

Demir, I. and Y. Samit. 2001. Seed quality in relation to fruit maturation and seed dry weight during development in tomato. Seed Science and Technology 29(2): 453-462.

Ellis, R.H., H.D. Hong and E.H. Roberts. 1985. Handbook of seed technology for genebanks No. 3. Volume II.

[5] "Zonificación de las especies de agraz (Vaccinium spp.) y una aproximación de su manejo agronómico como cultivos promisorios para la zona altoandina colombiana"

Compendium of specific germination information and test recommendations. International Board of Plant Genetic Resources. Chapter 36 Ericaceae. http://www.bioversityinternational.org/index.php?id=19&user_publications_pi1%5BshowUis%5D=2405; consulted: January, 2012.

Espinal, S. 1977. Zonas de vida: formaciones vegetales del departamento de Antioquia. Universidad Nacional de Colombia. Medellín, Colombia. 135 p.

Gaviria, C., C. Ochoa, N. Sánchez. C.I. Medina, M. Lobo, P. Galeano, A.M. Mosquera, A. Tamayo, Y. Lopera y B. Rojano. 2009a. Actividad antioxidante e inhibición de la peroxidación lipídica de extractos de frutos de mortiño (*Vaccinium meridionale* Swartz.) Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas 8(6): 519–528.

Gaviria, C., C. Ochoa, N. Sánchez, C.I. Medina, M. Lobo, P. Galeano, A.M. Mosquera, A. Tamayo, Y. Lopera y B. Rojano. 2009b. Propiedades antioxidantes de los frutos de agraz o mortiño (*Vaccinium meridionale* Swartz). pp. 93-109 In: Ligarreto, G. (ed.). Perspectivas del cultivo de agraz o mortiño (*Vaccinium meridionale* Swartz) en la zona altoandina de Colombia. Universidad Nacional de Colombia, Sede Bogotá, Bogotá. 134 p.

Giba, Z., D. Grubisic and R. Konjevic. 1995. The involvement of phytochrome in light-induced germination of blueberry seeds. Seed Science and Technology 23(1): 11-19.

Gómez, H. 1997. Estadística experimental con aplicaciones a las Ciencias Agrícolas. Universidad Nacional de Colombia, sede Medellín. Facultad de Ciencias Agropecuarias. 571 p.

Griffin, J.J. and F.A. Blazich. 2008. Vaccinium L. pp. 1154-1159. In: Bonner, F.T. and R.P. Karrfalt (eds.). Woody plant seed manual. U.S. Department of Agriculture Forest Service, Washington, D.C. 1223 p.

Haiwood, J.D. 1994. Seed viability of selected tree, shrub, and vine species stored in the field. New Forests 8(2):143-154.

Halliwell, B., R. Aeschbach, J. Loliger and O.I. Aruoma. 1995. The characterization of antioxidants. Food Chemical Toxicology 33(7): 601-617.

Harrington, J.F. 1972. Seed storage and longevity. pp. 145-245. In: Kolowsky, T.T. (ed.). Seed biology, 3 v. Academic Press, New York and London. 434 p.

Haywood, J.D. 1994. Seed viability of selected tree, shrub, and vine species stored in the field. New Forests 8(2): 143-154.

Heinonen, I.M. 2002. Antioxidants in fruits, berries and vegetables. pp. 23-51. In: Jongen W. (ed.). Fruit and vegetable processing. Woodhead Publishing Limited, Cambridge, England. 388 p.

Hernández, M.I. 2008. Estudio morfo-fisiológico de la semilla de mortiño (*Vaccinium meridionale* Swartz). Tesis Magister en Ciencias Agrarias. Facultad de Ciencias Agropecuarias. Universidad Nacional de Colombia, Medellín. 70 p.

Hernández, M.I. M. Lobo, C.I. Medina, J.R. Cartagena y O. Delgado. 2009. Comportamiento de la germinación y categorización y la latencia en semillas de mortiño (*Vaccinium meridionale* Swartz). Agronomía Colombiana 27(1): 15-23.

Hill, N.M. and S.P. Vander Kloet. 2005. Longevity of experimentally buried seed in *Vaccinium*: relationship to climate, reproductive factors and natural seed banks. Journal of Ecology 93(6): 1167-1176.

Hong, T.D. and R.H. Ellis. 1996. A protocol to determine seed storage behaviour. IPGRI Technical bulletin No.1. IPGRI, Rome. 64 p.

Jump, A.S., R. Marchant and J. Peñuelas. 2009. Environmental change and the option value of genetic diversity. Trends in Plant Science 14(1): 51-58.

Joseph, J., A.B. Shukitt-Hale, N.A. Desinova, D. Bielinski, A. Martin, J.J. McEwen and P.C. Bickford. 1999. Reversals of age-related declines in neuronal signal transduction, cognitive and motor behavioral deficits with blueberry, spinach, or strawberry dietary supplementation. Journal of Neuroscience 19(18): 8114-8121.

Khatun, A., G. Kabir and M.A. Bhuiyan. 2009. Effect of harvesting stages on the seed quality of lentil (*Lens culinaris* L.) during storage. Bangladesh Journal Agricultural Research 34(4): 565-576.

Kole, S. and K. Gupta. 1982. The timing of physiological maturity of seeds of sunflower: evaluation through multiple tests. Seed Science and Technology 10(3): 457-467.

Ligarreto, G.A., M.P. Patiño and S.V. Magnitskiy. 2011. Phenotypic plasticity of *Vaccinium meridionale* (Ericaceae) in wild populations of mountain forests in Colombia. Revista Biología Tropical 59(2): 569-583.

Lobo, M., J. Correa, G. Pérez y J. Arboleda. 1984. Madurez fisiológica de las semillas de tomate. Revista ICA 17(3):297-300.

Lobo, M. 2006. Recursos genéticos y mejoramiento de frutales andinos: una visión conceptual. Revista CORPOICA – Ciencia y Tecnología Agropecuaria 7(2):40-54.

Luteyn, J.L. 2002. Diversity, adaptation, and endemism in neotropical Ericaceae: Biogeographical patterns in the Vaccinieae. The Botanical Review 68(1): 55-87.

Mahesha, C.R., A.S. Channaveeraswami, M. B. Kurdikeri, M. Shekhargouda and M.N. Merwade. 2001. Seed maturation studies in sunflower genotypes. Seed Research 29(1): 95-97.

Matchett, M.D., S.L. MacKinnon, M.I. Sweeney, K.T. Gottschall-Pass and R.A. Horta. 2005. Blueberry flavonoids inhibit matrix metalloproteinase activity in DU145 human prostate cancer cells. Biochemical Cell Biology 83: 637-643.

Medina, C.I. 2010. Variabilidad poblacional y ecofisiológica del mortiño (*Vaccinium meridionale* Sw.), especie con potencial productivo y agroexportador en el trópico altoandino. Tesis Doctorado en Ciencias Agrarias. Universidad Nacional de Colombia, Facultad de Ciencias Agropecuarias, Medellín. 286 p.

Medina, C.I., M. Vargas, M. Lobo y J.L. Toro. 2008. La propagación sexual del mortiño. Corpoica, Corantioquia Plegable Divulgativo No. 61.

Medina, C.I., M. Lobo, M. P. Patiño, G.A. Ligarreto, O. Delgado, S.A. Lopera y J.L. Toro. 2009. Variabilidad morfológica en agraz o mortiño (*Vaccinium meridionale* Swartz) en la zona altoandina de Colombia. pp. 57-74. In: Ligarreto G. (ed.). Perspectivas del cultivo de agraz o mortiño (*Vaccinium meridionale* Swartz) en la zona altoandina de Colombia. Universidad Nacional de Colombia, sede Bogotá. Bogotá. 134 P.

Oladiran, J.A. and P.P. Kortse. 2002. Variation in germination and longevity of pepper (*Capsicum annum* L.) seed harvested at different stages of maturation. Acta Agronomica Hungarica 50(2): 157-162.

Petrov B., M. Doikova and D. Popova. 1981. Studies on the quality of eggplant seed. Acta Horticulturae 111: 273-280.

Ranwala, S.M. and R.E. Naylor. 2006. Production, survival and germination of bilberry (*Vaccinium myrtillus* L.) seeds. Botanical Journal of Scotland 56(1): 55-63.

Saastamoinen, O., K. Kangas and H. Aho. 2000. The picking of wild berries in Finland in 1997 and 1998. Scandinavian Journal of Forest Research 15: 645-650.

Shafii, B. and D.L. Barney. 2001. Drying and cold storage affect germination of Black Huckleberry seeds. HortScience 36(1): 145–147.

Shahram, S. 2007. Seed dormancy and germination of *Vaccinium arctostaphylos* L. International Journal of Botany 3(3): 307-311.

Shivashankaragouda B.P., M.N. Merwade and B.S. Vyakaranahal. 2007. Effect of harvesting stages, seed extraction and drying methods on seed quality of Brinjal Hybrid. Crop Research –Hisar- 33(1,2,3): 129-133.

Tanner, E.V.J. 1982. Species diversity and reproductive mechanisms in Jamaican trees. Biological Journal of Linnean Society 18(3): 263-278.

Wilbur, R.L. and J.L. Luteyn. 2008. A synopsis of the Mexican and Central American species of *Vaccinium* (Ericaceae). Journal of the Botanical Research Institute of Texas 2(1): 207-241.

Zuluaga, G.P. 2005. Dinámicas territoriales en frontera rural-urbana en el corregimiento de Santa Elena, Medellín. Escuela del Habitat, Facultad de Arquitectura, Universidad Nacional de Colombia, sede Medellín. 197 p.