Standardizing Vegetative Propagation as a Tool for Polycross Mating of Ascending Purple Milkvetch (*Astragalus adsurgens* Pall.)

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Abstract

This article highlights the efficacy of plant growth regulators and nutrient solutions on root development and survival of ascending purple milkvetch (APMV) cuttings. Three auxin-like growth regulators and Hoagland solutions were used as root inducing agents. The cuttings were planted in sand medium that allowed evaluation of rooting effectiveness. The study showed that up to 100% establishment of APMV cuttings can be achieved with the application of half-strength modified Hoagland (MHL) solution and plant growth regulators such as indole-3-acetic acid (IAA), 1-naphthaleneacetic acid (NAA) and indole-3-butyric acid (IBA) at 100mg L^{-1} strength. To the best knowledge of the authors, this is first time documentation of the vegetative propagation of APMV.

Introduction

APMV is a promising perennial forage legume for semi-arid prairies of Canada. It thrives in arid and semiarid temperate regions (Suttie, undated) with less amount of soil water than alfalfa (Guan et al., 2013). APMV plantation increases soil nitrogen and organic matter content while improving soil porosity and bulk density (Wang and Wang, 2013) and has been promoted for restoring degraded lands in China (Suttie, undated, Guan et al., 2013).

Preliminary results of a comparative study on five *Astragalus* species conducted at the Semiarid Prairie Agricultural Research Centre (SPARC) of Agriculture and Agri-Food Canada (AAFC) near Swift Current, Saskatchewan showed that APMV had comparable biomass yield and forage quality to that of cicer milkvetch (cv. AC Oxley II). The APMV was found frequently grazed by wild herbivore, which indicates its grazing preference. However, because of its relatively late maturing growth habit (Suttie, undated) compounded by wetter growing season and frequent grazing of terminal shoots by wildlife, it failed to reach the physiological maturity to produce seed prior to killing frost.

The propagation study was initiated with two objectives: rescuing the selected plants from the field and population improvement through polycross scheme. The study showed that APMV can be propagated from hardwood, semi-hardwood and top shoot cuttings with various degree of success. This article presents the results of top shoot cuttings propagated on the sand medium with the application of various rooting solutions.

Materials & Methods

The study was conducted at the SPARC of AAFC, near Swift Current, Saskatchewan in 2014-15. Initially, hardwood, semi-hardwood and top shoot cuttings were tested under various rooting treatments on a solid foam media (Oasis Horticubes Growing Media, Smithers-Oasis North America) in a growth chamber (Controlled Environments Ltd., Winnipeg, MB), with differential success. The rooted cuttings were then established in potted soil medium containing equal proportion of top soil, vermiculite and Professional Growing Mix (Sun Gro Horticulture). Top shoot cuttings were taken from the regenerated potted plants and further evaluated with five different root inducing treatments. The treatments involved 4-hour dipping of cut ends in the growth regulator solutions including IAA 100mg L^{-1}, NAA 100mg L^{-1}, IBA 100mg L^{-1}, mixture of equal strength solutions (100mg L^{-1}) of IAA, NAA and IBA, and half-strength MHL solution. The experiment was conducted in randomized complete block design with 3 replicates. Each treatment contained 6 to 11 cuttings.

The treated cuttings were planted in sand medium in 3”X3” size plastic containers with shallow layers of vermiculite and calcined clay put at the bottom and top of the container, respectively (Figure 1). The potted cuttings were put in propagator trays with clear transparent dome-shaped cover having two adjustable slits for air exchange.

Figure 1: A snapshot of experimental set-up

The growth condition in the greenhouse comprised 21°C/19°C day/night temperature with 300 µmol $m^{-2}s^{-1}$ of photosynthetically active radiation (PAR) for 16 hours a day. The sand medium was kept moist throughout the experiment by applying half-strength MHL solution. The tray cover was removed after 3 weeks to acclimate the plants under ambient humidity conditions. Upon removal of the cover, the plants were kept under lower light (100 µmol $m^{-2}s^{-1}$ PAR) for 3 days to prevent young leaves from photo-oxidative damage.

The cuttings were assessed for their survival and rooting percentage, root vigour and longest root length after 4 weeks of propagation. The root vigour was rated on a 1-5 score scale, considering both profuseness and length of roots. SAS 9.4 Proc Mixed Model was used for analysis of variance. When the F-tests were significant at $\alpha \leq 0.05$, the treatment means were compared with LSD at $\alpha = 0.05$.

Results and Discussion

Vegetative propagation is one of the essential tools for breeding perennial species (Mullin & Lee, 2013; Haissig et al., 1987). To the best knowledge of the authors, there is no prior information on the vegetative propagation of APMV. In a study carried out on cicer milkvetch, Başalma et al. (2008) reported a high survival and rooting success of hypocotyl and cotyledon explants using half strength Murashige and Skoog medium supplemented with 0.25 and 0.50 mg L^{-1} NAA. Efficacy of auxin-like growth regulators for inducing adventitious roots is well established (Pop et al., 2011). However, differential responses of plant species to different root inducing treatments suggests a complex genetic control on root development (Haissig et al. 1992).

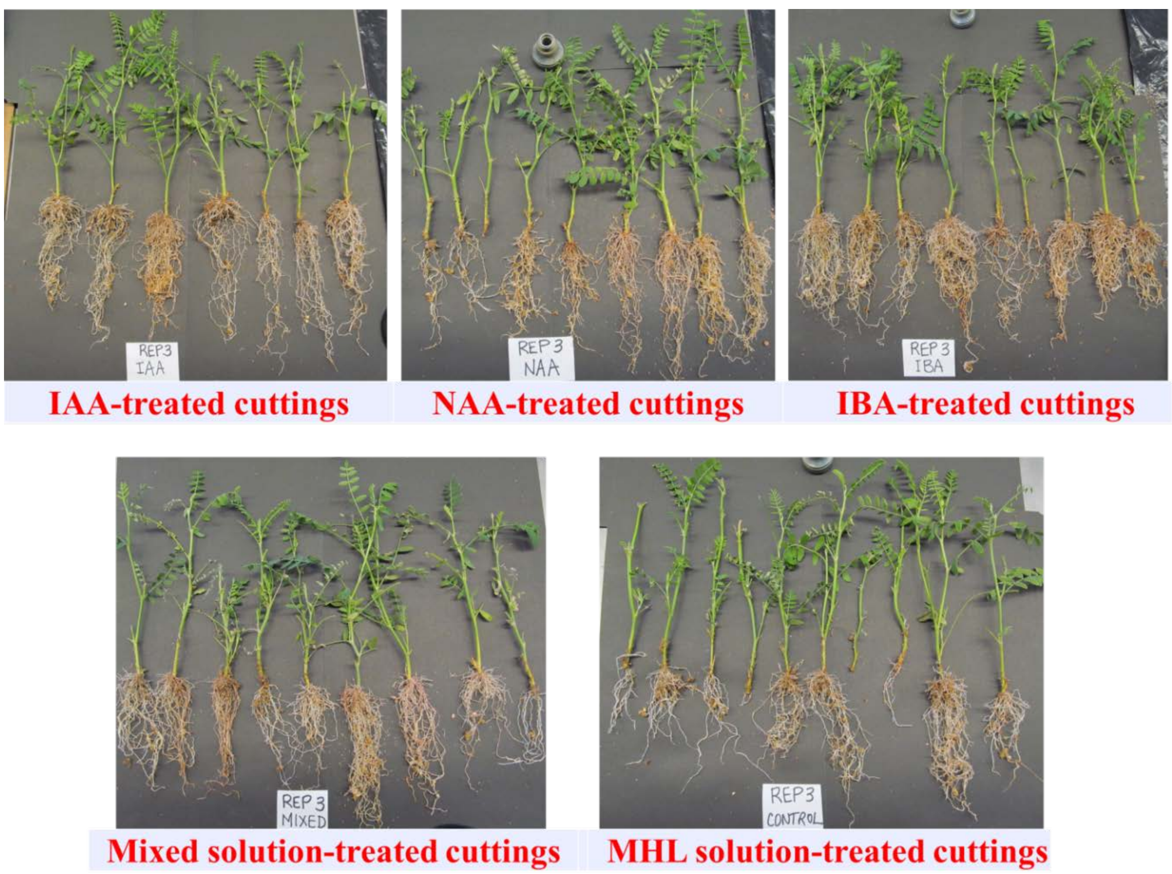

Figure 2: Root development in cuttings under different treatments in one of the replicates.

A preliminary study showed that APMV can be propagated from hardwood, semi-hardwood and top shoot cuttings with various degree of success. Initially, semi-hardwood cuttings had better survival than hardwood and top shoot cuttings while planted in the Oasis Horticubes foam medium in the growth chamber. However, some semi-hardwood cuttings developed die-back from the top, while the top shoot cuttings showed better persistence upon transplanting in the soil medium (data not shown). Building on this experience, top shoot cuttings were further assessed in the sand-based medium so as to evaluate the effectiveness of rooting. In this test, 4-hour dipping in IAA, NAA, IBA solutions, their mixture and MHL solution exhibited similar and high degree of effectiveness for plant survival, rooting percentage and average root length (Figure 2 &3). Growth regulator treatments produced significantly better root vigour than the MHL solution (Figure 3).

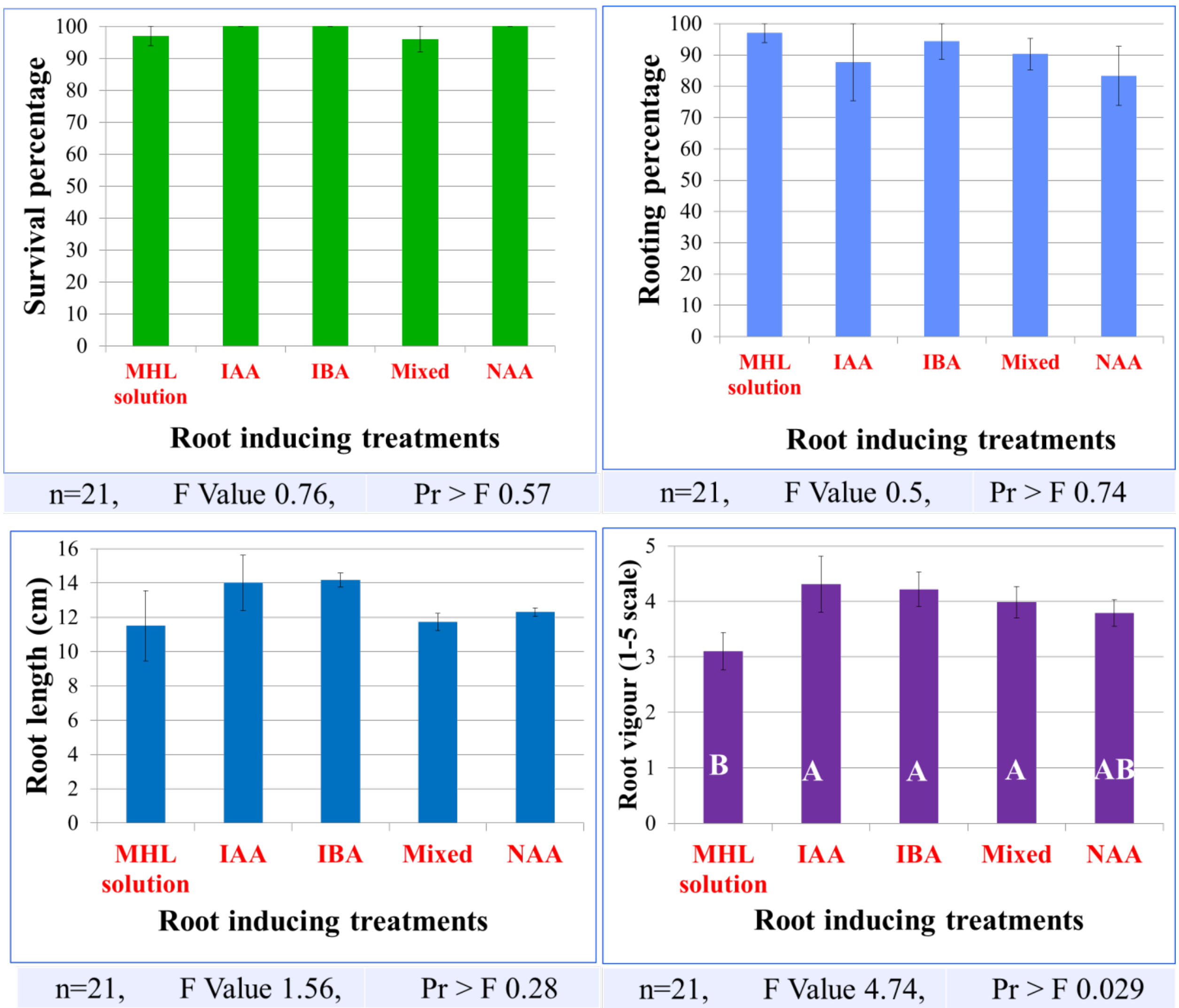

Figure 3: Plant survival and root parameters of the propagated cuttings after 4 weeks of propagation. The bars with different letters indicate significantly different means. The error bars are standard errors.

Conclusions

APMV can be propagated in sand-based medium in the greenhouse condition with high degree of success by using auxin-like growth regulators and half strength MHL solution. The growth regulator treatments produced better root vigour then the MHL solution. With this propagation tool in hand, efforts should be directed in developing early maturing APMV population for commercial uptake of the productive synthetic cultivars. Further study is also needed to determine the efficacy of agronomic and growth regulation measures for its commercial seed production.

References

Başalma, D.; Uranbey S.; Gürlek, D. and Özcan, S. (2008). TDZ-induced plant regeneration in *Astragalus cicer* L. African Journal of Biotechnology, 7 (8), 955-959.

Guan, X. K., Zhang, X. H., Turner, N. C., Xu, B. C., & Li, F. M. (2013). Two perennial legumes (*Astragalus adsurgens* Pall. and *Lespedeza davurica* S.) adapted to semiarid environments are not as productive as lucerne (Medicago sativa L.), but use less water. Grass and Forage Science, 68(3), 469-478.

Haissig, B. E., Davis, T. D. and Riemenschneider. D. E. (1992). Researching the controls of adventitious rooting. Physiologia Plantarum. 84 (2) 310-317.

Haissig, B. E., Nelson, N. D. & Kidd, G. H. (1987). Trends in the use of tissue culture in forest improvement. Nature Biotechnology 5, 52 – 59.

Mullin, T.J., & LEE, S.J. (2013) Best Practice for Tree Breeding in Europe. SKOGFORSK. Uppsala Science Park, 751 83 Uppsala.

Pop, T. I., Pamfil, D., & Bellini, C. (2011). Auxin control in the formation of adventitious roots. Notulae Botanicae, Horti Agrobotanici, Cluj-Napoca, 39(1), 307-316.

Suttie J.M. (undated). *Astragalus adsurgens* Pallas. http://www.fao.org/ag/agp/AGPC/doc/Gbase/DATA/pf000464.HTM, accessed 24 March 2015.

Wang, Z. B., & Wang, Q. Y. (2013). Cultivating erect milkvetch (*Astragalus* adsurgens Pall.) (Leguminosae) improved soil properties in loess hilly and gullies in China. Journal of Integrative Agriculture, 12 (9), 1652-1658.

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