EFFECT OF CUTTING POSITION IN THE MOTHER PLANT AND USE OF AUXIN ON THE ROOTING EFFICIENCY OF CUTTINGS IN FOUR VARIETIES OF *CANNABIS SP.*

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Abstract. The regulatory framework for the production, industrialization, and commercialization chain of Cannabis sp. plant, its seeds, and derived products for industrial and/or medicinal use has recently been approved in Argentina. This work addresses the importance of producing genetically stable plant material, especially in medicinal production where cloning plants with known characteristics is essential for large-scale production. The study analyzed the influence of cutting position on the mother plant and the use of rooting agent on the rooting ability of four varieties Cannabis sativa L. Significant differences were found between genotypes evaluated for their rooting ability, although no clear effect was determined from positioning cuttings on different parts mother plants. It is concluded that there is a varietal component to observed response that can change with or without addition rooting agent, mainly affecting root length and weight. These findings suggest that growers could select rooting techniques that maximize success across some variety cultivars while eliminating those difficult to root from their production systems.

Keywords: vegetative propagation, macro propagation, helm, improvement

INTRODUCTION

Cannabis sativa L. is an herbaceous plant, mainly dioecious, with female and male inflorescences in separate individuals, allogamous, cross-pollinated mainly by wind, with a short daytime photoperiodic response, belonging to the family Cannabaceae (Lu, X.; Clarke, R.C. 1995). Archaeological evidence supports that this plant has been cultivated by humans for more than 6000 years (Lu, X.; Clarke, R.C. 1995) and it is estimated that it originated in the north-eastern Tibetan plateau where it was domesticated to obtain fibre from its stems, oil from its seeds and resin from its epidermal glands (Dewey, L.H. Hemp. 1914; Miller, N.G. 1970; Small, E. 1975). There are different botanical varieties that differ in the production of cannabinoids and in the content and quality of fibres in their stems. The term Cannabis is commonly used for plants whose main production is the various cannabinoids and Hemp for fibre plants. Hemp is currently an incipient business in international trade; France and China are the main producers and the Czech Republic is the main importer (SAGyP, 2022.). On the other hand, the recent legalization of Cannabis sp. for medicinal purposes in many regions - such as Argentina - has revealed the need for genetic improvement, cultivation and propagation adjustment for the species.

The regulatory framework for the production, industrialization and commercialization chain of the Cannabis plant, its seeds and derived products for industrial and/or medicinal use has recently been approved in Argentina. This crop could be incorporated into production systems as an alternative to summer crops in extensive plantations and to horticultural crops in intensive plantations, for which it is essential to obtain agronomic information. Although in Argentina, the National Seed Institute (INASE) has begun to register local varieties through the National Register of Cultivars and Cultivar Ownership, the commercialization of controlled seeds is still limited in terms of scale production. In this sense, vegetative propagation (macro or micro) is indispensable for plant material that ensures the genetic stability of a given variety (Caplan et al, 2018; Bosco et al, 2022; Mejía-Londoño et al, 2023). Macro propagation by cuttings is a low-cost method applied to various plants such as Cannabis that produces genetically uniform plants7. The advantages of the technique, in addition to the genetic stability of species of interest, is the achievement of high multiplication rates in a sustainable way and the possibility of combining it with *in vitro* culture methods (Bosco et al.2022: Jiménez-Terry & Agramonte 2013). However, studies have been reported in which, when attempting to propagate *Cannabis* vegetative, it was observed that rooting uniformity and rooting success varied widely (Cockson et al, 2019). The difference in the ability of cuttings to form adventitious roots often depends on the maturity of the mother plant (Schreiber & Kawase1975; Morgan & Mcwilliams 1976). Cuttings from juvenile plants generally have better rooting than those from mature plants in woody species and this is because juvenile plant material sometimes has a higher content of endogenous auxins and other rooting promoters compared to mature material (Husen &Pal 2006; Altamura 1996). Furthermore, in lignified cuttings, maturity often varies according to the position of the cutting (cutting position on the mother plant) on the mother plant; cuttings from basal areas often retain juvenile characteristics and have an enhanced ability to form adventitious roots (Hackett, 1970). There is limited information and diverse findings on the effects of cutting position or cutting position on the mother plant on adventitious rooting in herbaceous plants. Some authors report that the location of the cutting and the number of leaves per cutting can affect rooting success and vigour (Caplan et al.2018, Bosco et al.2022). On the other hand, the use of rooting promoters, either synthetic or natural, has been shown to influence propagation by Cannabis cuttings (Bosco et al, 2022; Campbell et al. 2021; Kurtz et al. 2022; Favero et al. 2023). Therefore, the aim of this study was to evaluate the influence of the position of the cutting in the mother plant (medial or apical) and the use of rooting promoter on the rooting capacity of four varieties of *Cannabis sativa* L. for medicinal use. We also sought to detect the incidence of the varietal component in the observed responses.

MATERIALS AND METHODS

Plant material

The mother plants were 4 cannabis individuals belonging to different varieties - Purple kush; Northern lights; Zombie kush and Haze (from now, A, B, C and D respectively). They were three months old and grown under non-flowering-inducing photoperiods (16 hours of light). From these plants, 10 branches measuring 20-25 cm in length were collected. This procedure was repeated on 3 occasions, with intervals of 20 days apart. The stem diameter varied between 2.5 and 3.5 mm (Figure 1). To obtain the cuttings, each branch was divided into two: the apical portion and the middle portion. This allowed us to obtain cuttings of about 10 cm in length with the apical shoot plus one leaf remaining. The leaf area was reduced to minimize evapotranspiration (Figure 2a and b).

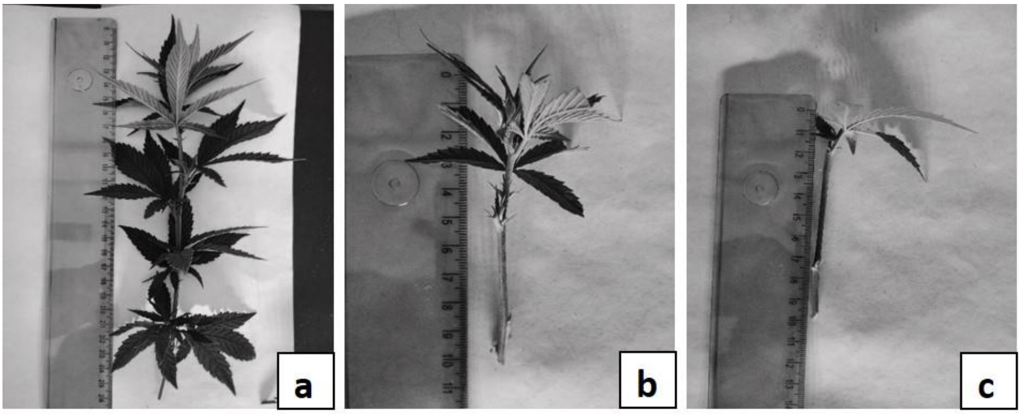

Fig. 1. Initial plant material- a. Branch from which the cuttings were obtained. b- Cuttings from apical c- Cuttings from medial position.

The cuttings were planted in metal trays measuring 60 cm x 55 cm x 15 cm (L x W x D), spaced 12 cm apart, using a commercial peat-based substrate with high moisture retention capacity (Terraferil® - Vitaflor® Light Mix). The substrate contains a large quantity of organic matter with 80-85% NO^3 concentration between 250-450 ppm, C/N ratio around 30-35, P content 25-100 ppm and K content 200-300 ppm; pH 5.00-5.80 and electrical conductivity ranged from 20 to.60 mS/cm. The cuttings displayed a fresh biomass of approximately 1 g. Before planting them in the substrate, half of each variety's cuttings (5) were treated with commercial auxin rooting gel (ANA-alpha-naphthalene acetic acid at a concentration of.1g per 100 cc), while the remaining half were directly planted without any growth regulator usage. Ambient humidity conditions were maintained between 75% and 83% RH, with temperature ranging from 23°C to 24°C. The humidity level was kept at field capacity values within the substrate while spraying water twice daily onto the cuttings. Lighting was provided by LED luminaires (Mars Led Sun flash 420) with a CRI rating of 95% and PPF value of 2.5 µmol/J).

A non-inductive photoperiod consisting of 16 hours' light was maintained indirectly. After 30 days, the cuttings were removed from the trays by washing the substrate, ensuring the preservation of the roots. The rooting capacity was assessed by calculating the ratio of rooted cuttings to total cuttings. For those cuttings that developed roots, the length of the longest root was measured up to the base of the plant. Subsequently, the cuttings were dried in an oven at 60°C until a constant weight was achieved, to determine the total biomass of the cuttings and root biomass.

Analysis of results. A factorial analysis of variance was conducted, considering the varieties, the position of the cutting on the mother plant, and the presence or absence of a rooting agent. Significant differences ($p<0.05$) were compared using the LSD Fisher test, and variance assumptions were verified. Infostat software (Di Rienzo et al., 2020) was utilized for the analysis.

RESULTS

Under the conditions evaluated in this study, all cuttings successfully developed roots. Nonetheless, variations were noted in the percentage of rooting, as well as in the quality, quantity, and length of roots among different varieties. As illustrated in Figure 2, discrepancies in root characteristics were evident across the tested varieties.

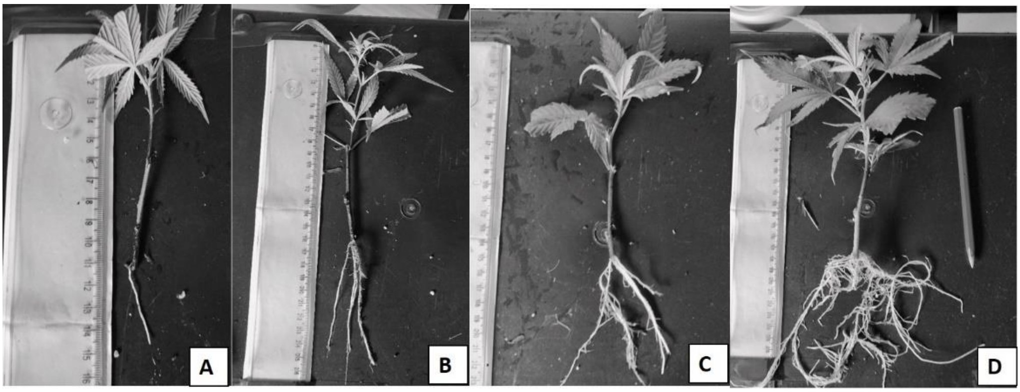

Fig. 2. Cuttings after 30 days in culture. Varieties A.B.C.D exhibit differences in root length, root type, and root hairs.

Significant discrepancies were identified among the four varieties regarding the percentage of root formation in the cuttings, as depicted in Figure 3.

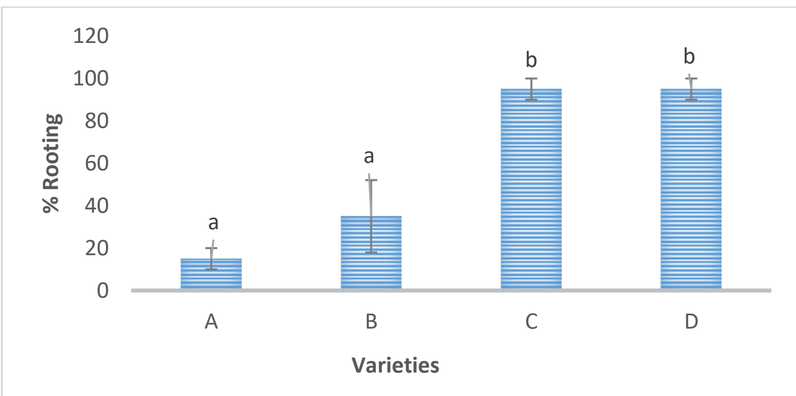

Fig. 3. Rooting efficiency, expressed as a percentage of the total, for the tested varieties A, B, C, and D. Lowercase letters denote statistically significant differences according to the LSD test ($p\leq 0.05$). The results obtained highlight the impact of genotypic variability on cutting rooting. Specifically, varieties A and B exhibited a lower rooting percentage under the conditions tested

No statistically significant differences were observed in rooting ability based on the cutting position on the mother plant effect Figure 4) or the use of growth regulators (ANA) (Figure 5), regardless of the variety.

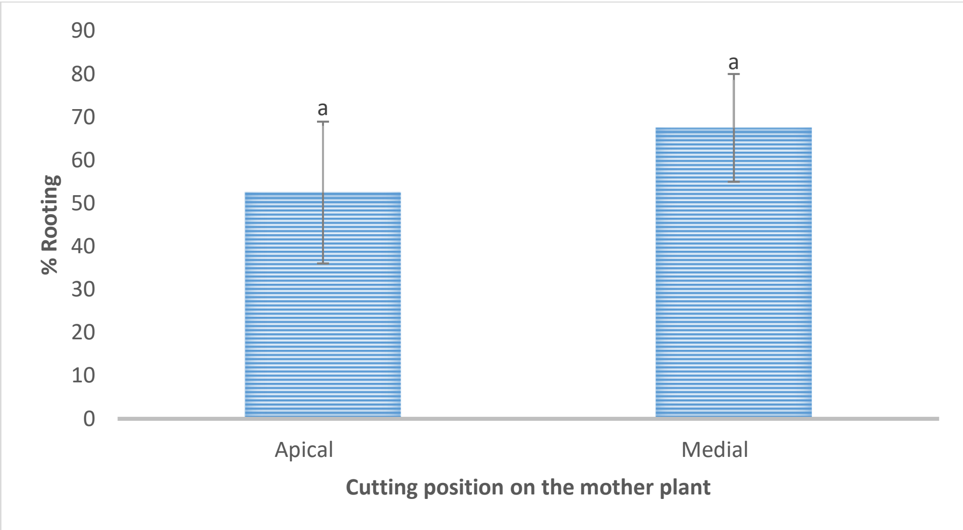

Fig. 3. Effect of cutting position on the mother plant (apical or medial position) on rooting ability, expressed as a percentage of the total. Different lowercase letters indicate statistically significant differences according to the LSD test ($p \leq 0.05$)

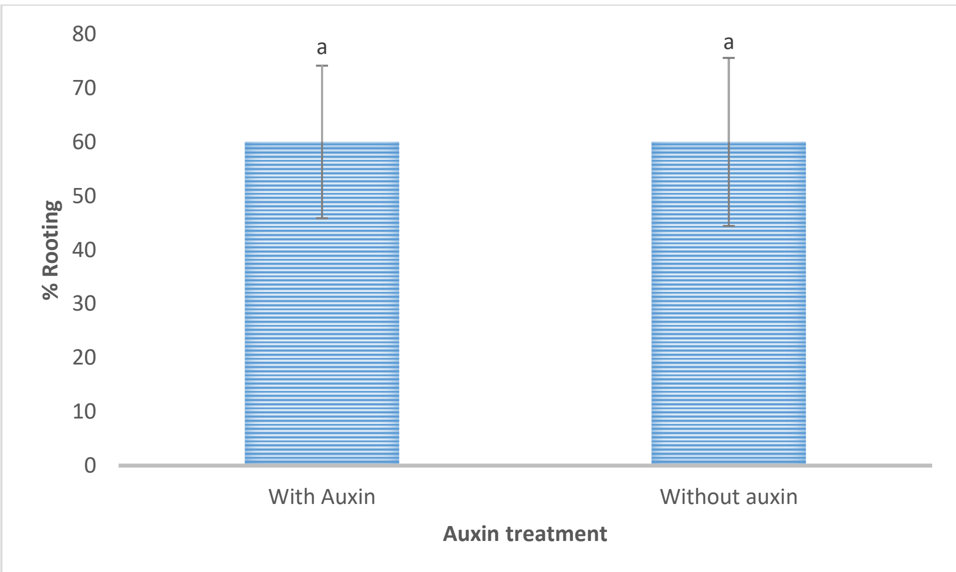

Fig. 5. Effect of auxin application on rooting efficiency -as % of total-. Lower case letters indicate statistically significant difference according to LSD test ($p \leq 0.05$)

Significant differences in root length, total dry biomass of cuttings, and dry biomass of roots were found according to variety (Table 1).

Table 1

Root length (cm), cutting dry biomass (g) and root dry biomass (mg) of the tested varieties A-B-C-D. Lower case letters indicate statistically significant differences according to the LSD test ($p \leq 0.05$)

Variety	Roots length	Total biomass of cuttings	Root biomass
A	7,6 a	350 a	20,4 a
B	7,0 a	330 a	7,5 a
C	17,83 b	500 ab	48,0 ab
D	22,58 b	660 b	72,1 b

In addition, an interaction between the position of the cutting on the mother plant (cutting position on the mother plant) and the varieties was found in root length, as shown in Figure 6.

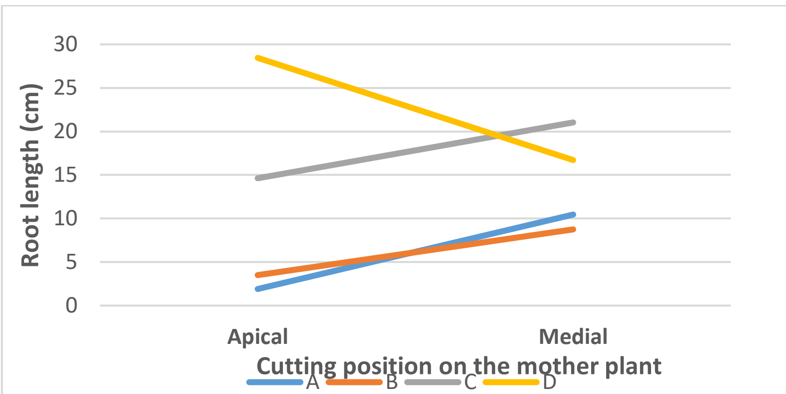

Fig. 6. Interaction between cutting position on the mother plant and variety in root length. Varieties A, B, and C exhibited greater root length in cuttings from the middle part, while cuttings from the apical portion of Variety D showed greater length

Regarding root length, total biomass of cuttings, and root biomass, we observed very low values for these parameters in varieties A and B. Therefore, we analysed whether there was a significant difference in these parameters only in varieties C and D. As shown in Figure 7, both root length and total dry biomass of cuttings showed significant differences, with an increase in both parameters observed when a rooting agent was used.

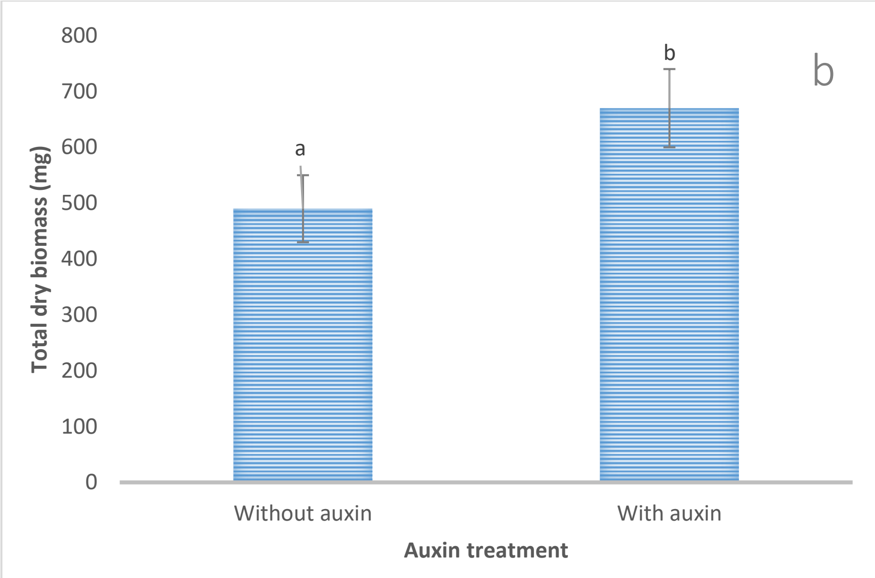

Fig. 7 a. Root length with and without the use of an auxinic rooting agent. b.Total dry biomass of the cutting with and without the use of an auxinic rooting agent. Different lowercase letters above the columns indicate statistically significant differences according to the LSD test ($p \leq 0.05$)

DISCUSSION

Significant differences were found in the rooting capacity of each genotype. Regarding the genotypic variability in rooting efficiency, other authors (Campbell et al., 2021) also observed significant variation in rooting response among the cultivars they evaluated. Caplan et al. (2018) found a significant interaction between cultivar and hormone application, indicating that the optimal method of hormone application and auxin concentrations may vary between cultivars or varieties. Genetics affected the overall rooting success in this study. Comparable results have been recorded for Cannabis cultivars 'Ghost Train Haze,' 'Bubba Kush,' and 'Headband,' with rooting rates of 85%, 40%, and 40%, respectively (Campbell et al., 2019). The rooting differential identified in the literature and observed in this study is cultivar-specific but may be influenced by environmental and treatment conditions (Hartmann et al., 2014). Proper selection of genetics is a critical consideration for large-scale growers who demand a stable rooting response (Campbell et al., 2019). There is limited information and conflicting results on the effects of scion position on the mother plant on adventitious rooting in Cannabis plants. Some studies indicate that the position of the cutting does not influence the rooting success rate or the quality of the roots (Caplan et al., 2018). In our work, no differences were observed between medial and apical cuttings with respect to rooting success or quality. These results suggest that, in certain Cannabis varieties, cutting position does not play an important role in rooting. Similar results were found in tea stem cuttings (Soundy et al., 2008), which could be attributed to the absence of distinct maturation stages in herbaceous plants, in contrast to woodier species (Caplan et al., 2018). In contrast, other studies report that the location of the branch on the mother plant is a determinant for increasing the rooting percentage, with basal cuttings rooting at a significantly higher rate than apical cuttings. However, they did not explain the cause of this difference (Bosco et al., 2022).

Regarding the use of ANA, the results of this study are in agreement with those obtained by other authors (McLeod et al., 2022), who showed that growth regulators have no effect on the rooting of Cannabis sativa 'I3' cuttings. According to research by Campbell et al. (2021), rooting hormone selection had a minimal impact on rooting success compared to cultivar and medium selection, although a significant improvement was achieved when a hormone was used versus the control. These authors evaluated eight commercial Cannabis cultivars and found that rooting success was significantly improved with the application of growth regulators (IBA, ANA, and other organic compounds, alone or in different combinations) in all cultivars. Cuttings subjected to synthetic IBA hormone treatments formed a higher number of roots with better quality in other investigations (Caplan et al., 2018; Kurtz et al., 2022). In trials of IBA-treated cuttings, rooting performance was higher, with an increase in root length, root dry weight, and root-to-shoot dry weight ratio compared to water-treated cuttings (Favero et al., 2023). It is documented that treatment of the basal portions of stem cuttings with synthetic auxins such as IBA or ANA can improve the rooting success rate, increase the speed of rooting, and increase the amount of adventitious roots in various plants (Hartmann et al., 2014). However, their use is restricted in organic productions. Therefore, alternative hormones or techniques are used to improve the success rate and quality of rooting. The use of powder from the invasive algae *U. pinnatifida* proved to be as effective as ANA, making it an excellent alternative for asexual propagation in organic Cannabis production, while also addressing the issue of using a species with negative effects on the marine ecosystem (Bosco et al., 2022). Although an interaction between cutting position on the mother plant and root length was found in this work, no references to this relationship were found in the literature. Some reports indicate that the position (apical or basal) from which *C. sativa* cuttings were taken had little effect on rooting success or quality. However, the application of a 2,000 ppm IBA treatment showed a 2.1 times higher rooting success rate and 1.6 times higher root quality compared to a 2,000 ppm willow (*Salix alba* L.) extract. In other species, the influence of the size and position of the cutting on root quality has been reported. For example, in a study on the halophytic species S. verrucosum, cuttings from the mid-apical portion obtained the highest percentage of root number and fresh and dry weight (Lastiri-Hernández & Álvarez-Bernal, 2020). Similarly, in *Rosmarinus officinalis* L., the fresh and dry biomass of roots is mainly influenced by the size of the cutting. Cuttings from the mid-apical portion had the highest percentage of rooting, number of roots, fresh and dry weight of both root and plant area, stem diameter, and plant height (Álvarez-Herrera et al., 2007)."

CONCLUSIONS

In this work, statistically significant differences at the 5% level were observed between the genotypes tested for the rooting ability of cuttings. No effect of cutting position on the mother plant on these differences was determined. The results indicate that there is a varietal component in the observed rooting response, which can be influenced by the addition of a rooting agent, affecting root length and root weight.

These findings are relevant for production objectives involving the genetic stability of Cannabis. Growers could benefit by applying a single rooting technique or a limited number of techniques, achieving high rooting success across a wide range of cultivars and eliminating those that are difficult to root from their systems

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