

Somatic-embryogenesis derived leaf-rust-tolerant clones of arabica coffee to deal with climate change

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Abstract. The high-altitude coffee growing, Arabica, is likely subject to the global warming effect as they are prone to leaf-rust attacks at a higher temperature. It supplied 70% of world coffee production for its popularity concerning its delicacy and aromatic flavor. Utilization of, genetically, superior planting materials, i.e. leaf-rust-tolerant Arabica, has become an essential point, as may provide the potential solution to prevent the lost production due to leaf-rust attack. Andungsari 2K (AS 2K), S795, AS1, and Sigararutang are some of the potential leaf-rust-tolerant Arabica clones in Indonesia. Vegetative propagation by somatic embryogenesis may support the availability of superior plant materials quickly. The major aim of this experiment was to study the effect of different clones on germination step after the preliminary stage of direct-embryogenesis from leaf explants with combinations of medium between auxin (2,4-D) and cytokinin (2-ip). Embryo germination stage where embryooid was transferred to the germination medium consisting of MS medium without hormones. The results revealed that the growth location and texture of callus, as well as growth patterns and colour of embryogenic callus, were significantly influenced through the different combinations of medium and clones. The clone of S795 exhibits the highest embryo germination percentage of up to 100% within 8 weeks experiment period.

1 Introduction

World wide changes against a biotechnology allocate new requirements for coffee commodity. The utilization of coffee plant material which adapted to the impact of climate change is one of the alternative solutions. Rising temperatures and erratic rainfall patterns pose a major risk to the future of coffee production in producing countries such as Indonesia. The planting material of coffee leaf rust tolerance is one of the alternative ways to deal with climate change. Arabica coffee which popular with its soft and unique taste contributed to 70% of world coffee production. Nevertheless, it is not tolerance with pest and disease [1] and should be planted in high land above 1200 m deep sea level. The utilization of genetically superior planting materials becomes essential for expanding biomass productivity. Leaf rust tolerant of Arabica becomes the most preferred choice due to effective results. Andungsari 1 (AS1), Andungsari 2K (AS 2K), S795 and Sigararutang are some of the commercial tolerant Arabica varieties in Indonesia. Vegetative propagation by somatic embryogenesis of those varieties supports a prospective distribution system for this purpose. Somatic embryogenesis has been successfully achieved for scale-up production in *C. arabica* species [2, 3, 4]. There have been several somatic embryogenesis in *C. arabica* [5-16]. This method has been accomplished by direct somatic embryogenesis from globular without callus formation or

by indirect somatic embryogenesis through friable embryogenic callus proliferation [17].

The fundamental purpose of this research was to compare the response of germination step from the preliminary step of direct-embryogenesis from leaves explants of some Arabica varieties leaf rust tolerant. The response between plant growth regulator and varieties to induce callus, qualitative characteristic embryogenic calli and embryo germination responses were studied.

2 Materials and Methods

A completely randomized design of laboratory observation was carried out with coffee clones, i.e. AS2K, S795, AS1, and Sigararutang as the first factor, and 8 levels of medium treatments, i.e. 2-ip and 2,4-D for the second factor. Leaf rust tolerant varieties of Arabica coffee were subjected to chemical treatments to induce and express the embryogenic callus, based on protocol reported elsewhere [18]. The eight combination of mediums are M1 (1 mg/L 2-ip and 0.2 mg/L 2,4-D), M2 (1 mg/L 2-ip and 1 mg/L 2,4-D), M3 (2 mg/L 2-ip and 0.2 mg/L 2,4-D), M4 (2 mg/L 2-ip and 1 mg/L 2,4-D), M5 (3 mg/L 2-ip and 0.2 mg/L 2,4-D), M6 (3 mg/L 2-ip and 1 mg/L 2,4-D), M7 (4 mg/L 2-ip and 0.2 mg/L 2,4-D), and M8 (4 mg/L 2-ip and 1 mg/L 2,4-D). Qualitative characteristics of coffee clones as responses to a different medium for direct somatic embryogenesis

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were observed. The embryoid will be transferred to the germination medium consisting of MS [19] medium in the absence of a plant growth regulator. Furthermore, embryo germination, open cotyledonary, root length, hypocotyl length and the number of roots were subsequently observed, the analysis of variance was performed with further Tukey post-hoc analysis to reveal the interaction effect of clone and week.

3 Result and Discussion

The effect of medium treatments on some qualitative characteristics of coffee embryogenic calli is inconsistently varied among clones (Table 1). The coffee clones may determine callus location with all AS2K coffee observed in vein leaf while most of S795 in the entire leaf edge. While all Sigararutang coffee observed in the entire leaf edge. Both in vein leaf and entire leaf edge were showed for AS1 coffee clone. Furthermore, the slow growth of callus for AS2K may indicate the absence of a medium effect. It is different from S795 with callus response following medium treatments varying from slow to vigorous.

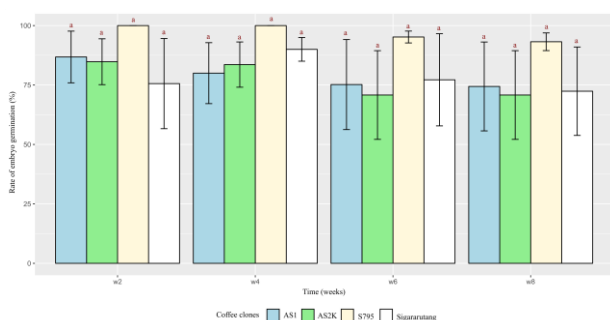


Fig. 1. Rate of embryo germination in different coffee clones

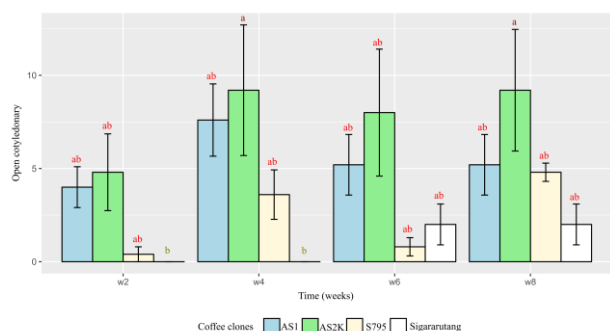


Fig. 2. Open cotyledonary in different coffee clones

The best performance was Sigararutang coffee clone which was moderate and vigorous. While most of the AS1 coffee clones exhibited slow responses. All these data may imply the importance of genetic factors and endogenous hormone posed in different coffee clones to characteristics of embryogenic calli. Type and colour of embryogenic calli were varied from brownish, yellowish and creamish between morphogenic and non morphogenic.

Table 1. Qualitative characteristics of coffee clones as responses to different medium for direct somatic embryogenesis (M: morphogenic; NM: non-morphogenic; SGR: Sigararutang; VI: Vein leaf; Ele: entire leaf edges)

No	Clones	Treat	Type	Colour	Loc	Growth
1	AS2K	M1	NM	Brownish	VI	Slow
2	AS2K	M2	M	Yellowish	VI	Slow
3	AS2K	M3	M	Creamish	VI	Slow
4	AS2K	M4	M	Yellowish	VI	Slow
5	AS2K	M5	NM	Brownish	VI	Slow
6	AS2K	M6	NM	Brownish	VI	Slow
7	AS2K	M7	NM	Brownish	VI	Slow
8	AS2K	M8	NM	Brownish	VI	Slow
9	S795	M1	M	Brownish	VI	Moderate
10	S795	M2	M	Brownish	VI	Vigorous
11	S795	M3	M	Creamish	Ele	Moderate
12	S795	M4	M	Creamish	Ele	Moderate
13	S795	M5	NM	Yellowish	Ele	Slow
14	S795	M6	M	Yellowish	Ele	Slow
15	S795	M7	NM	Creamish	Ele	Slow
16	S795	M8	NM	Yellowish	Ele	Slow
17	SGR	M1	M	Yellowish	Ele	Moderate
18	SGR	M2	M	Creamish	Ele	Vigorous
19	SGR	M3	M	Yellowish	Ele	Vigorous
20	SGR	M4	M	Yellowish	Ele	Moderate
21	SGR	M5	M	Yellowish	Ele	Moderate
22	SGR	M6	M	Yellowish	Ele	Moderate
23	SGR	M7	NM	Yellowish	Ele	Moderate
24	SGR	M8	M	Yellowish	Ele	Moderate
25	AS1	M1	NM	Brownish	VI	Slow
26	AS1	M2	M	Brownish	VI	Moderate
27	AS1	M3	NM	Creamish	Ele	Slow
28	AS1	M4	M	Yellowish	Ele	Slow
29	AS1	M5	NM	Yellowish	Ele	Slow
30	AS1	M6	NM	Yellowish	Ele	Slow
31	AS1	M7	NM	Brownish	VI	Slow
32	AS1	M8	NM	Brownish	VI	Slow

Each treatment showed different responses of callus induction and expression of embryogenic calli due to the effect of 2-ip and 2,4-D concentration ratio from preliminary step of direct embryogenesis. This difference is due to genetic factors among varieties. The results showed that AS2K and AS1 required high concentration of cytokinins and a small concentration of auxin for callus induction (M3 medium), while embryogenic callus formation requires cytokinins with low concentration and auxin with high concentration (M2 medium). S795 requires cytokinins and auxin with

high concentration for induction callus (M4 medium) but the formation of embryogenic callus requires cytokinins with low concentration and auxins with high concentration (M2 medium). The location and texture of callus were fundamentally influenced by the various combinations of medium and varieties [2]. Moreover, callus growth pattern and colour were also principally influenced through the presence of various combinations of medium and varieties [2].

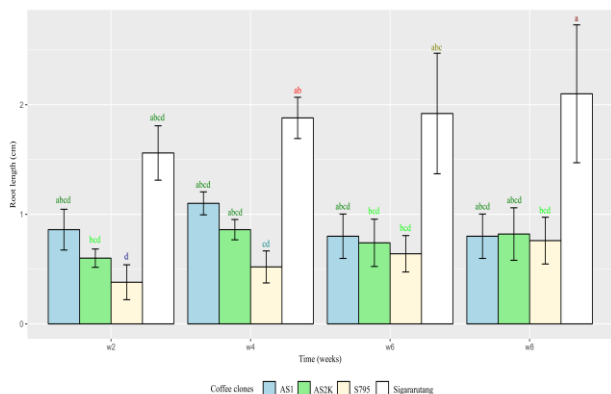


Fig. 3. Root length in different coffee clones

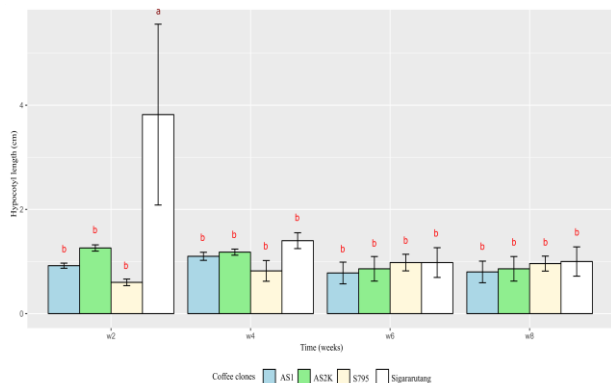


Fig. 4. Hypocotyl length in different coffee clones

The successful germination stage is the crucial step for the maintenance planlet before ready for the acclimatization step to avoid exaggerated plant losses in the nursery. The response of embryo germination was varied and it was largely determined by varieties. The highest percentage of embryo germination was S795 which accounts for 100% in four weeks (Figure 1). Every embryo has a different endogenous ability which is only the perfect and vigorous embryo can be survived longer in the further phase of somatic embryogenesis. An appropriate maturation embryo is a prerequisite as a beginning component to produce planlet derived from somatic embryos [20].

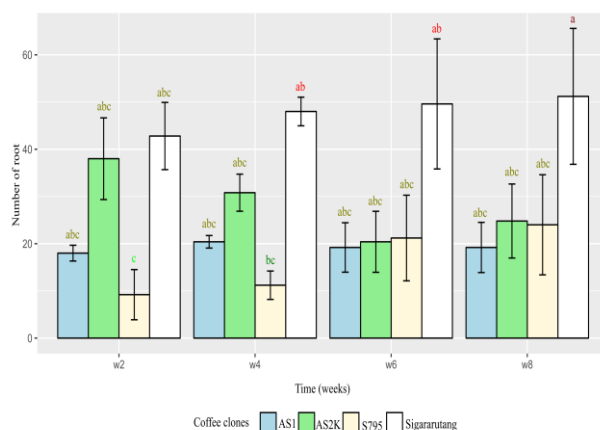


Fig. 5. Number of roots in different coffee clones

The accomplishment of planlet acclimatization was established by the proportion of open cotyledonary embryo (Figure 2). AS2K coffee clone was the highest number in the response of open cotyledonary embryo. It was significantly different compared with other clones. It means that AS2K's embryo better germination capacity because of mature physiology, even though the other variable parameters were still low. The highest number of open cotyledonary, the highest chance for shoot formation quickly. Sigarutang coffee clone has the longest root and hypocotyl every week as well as with the number of the root (Figure 3,4 and 5).

4 Conclusion

Every clone have a different response in a different medium for the characteristics of type, colour, location, and growth for direct somatic embryogenesis. The highest percentage of embryo germination is S795. Sigarutang has the longest root and hypocotyl as well as the number of root formation. AS2K coffee clone was the highest number in the response of open cotyledonary embryo. This different response between parameters was largely determined by variety.

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