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Imbibition curve of oil radish seeds

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Abstract: Tried to establish the duration of phases in the imbibition process of oil radish seeds, seeds of cultivar CATI AL-1000 lot of 2004, and the cultivars IPR 116 lot of 2004 and 2005 were soaked in "gerbox" box, under moistened paper in Biochemical Oxygen Demand (B.O.D.) adjusted in temperature 25°C, with four replications of 50 seeds. Was calculated the percentual increment of mass over the time, in function of initial mass in the intervals of time of 15 minutes, 2 hours, 4 hours, 6 hours and 12 hours. The research was conducted in two experiments, the first with the same sample of seeds in the weighings and the second with different samples. In the first experiment, the cultivar CATI AL-1000, ended the phase I with 28,55 hours and the phase II with 35,87 hours. Cultivar IPR 116 ended the phase I with approximately 18,05 hours for the lot of 2004 and 21,32 for the lot of 2005, beginning from this point, the phase III. In the 2nd experiment, seeds of cultivar CATI AL-1000 concluded the phase I with 49,07 hours and the phase II with 116,93 hours. For seeds of cultivar IPR 116, lots of 2004 and 2005, was not verified the phase II of imbibition, being the phase I finalized with 52,55 hours for the lot of 2004 and 152,78 hours for the lot of 2005 with the germination of seeds started after this period.

Keywords: *Raphanus sativus*, germination, water.

Curva de embebição de sementes de nabo forrageiro

Resumo: Procurou-se estabelecer duração das fases no processo de embebição de sementes de nabo forrageiro. A pesquisa foi conduzida em dois experimentos. O primeiro constituiu-se da mesma amostra de sementes nas pesagens e o segundo de amostras diferentes. Sementes da cultivar CATI AL-1000 do lote de 2004, e da cultivar IPR 116 lote de 2004 e 2005 foram colocadas para embeber sobre papel umedecido, a 25°C, em Biochemical Oxygen Demand (B.O.D.), com quatro repetições

de 50 sementes. O incremento porcentual de massa foi calculado em função da massa inicial das sementes nos intervalos de tempo de 15 minutos, 2, 4, 6 e 12 horas, sendo cada intervalo com duração de 12 horas. No 1° experimento, a cultivar CATI AL-1000 finaliza a fase I aproximadamente com 28,55 horas e a fase II com 35,87 horas. As sementes da cultivar IPR 116 finalizaram a fase I com aproximadamente 18,05 horas para o lote de 2004 e 21,32 horas para o lote de 2005, iniciando-se, a partir deste ponto a fase III. No 2° experimento, sementes da cultivar CATI AL-1000 concluíram a fase I com 49,07 horas e a fase II com 116,93 horas. Para as sementes da cultivar IPR 116, lotes de 2004 e 2005, não se verificou a fase II de embebição, sendo a fase I finalizada com 52,55 horas para o lote de 2004 e, para o lote de 2005, a 152,78 horas do início da embebição, com a germinação das sementes iniciada logo após este período.

Palavras-chave: *Raphanus sativus*, germinação, água.

Introduction

Fundamental for cellular metabolism during the germination, the water promotes the enzymatic activity and the degradation of reserve substances producing energy to the resumed of growth of embryo. To occur the germination, seeds need to achieve an adequate level of hydration that allows the reactivation of metabolism, contributing to the process of mobilization and assimilation of reserves and consequent growth of embryonic axis (Johnson-Flanagan & Nykiforuk, 1999).

This hydration or imbibition of water by seeds is a process purely physical, which varies from specie to specie, according with permeability of tegument, hydrical availability, hydrostatic pressure, temperature, contact area seed/water, intermolecular forces, physiological conditions of seeds and chemical composition. During the imbibition, must exist a gradient of potential hydrical and an affinity between the compounds (seeds and water).

This imbibition of water by seeds, under optimal conditions, occurs according a standard three-phase, where the phase I is a physical process of initial imbibition controlled mainly by the differences of matricial forces between the dry seeds and the water in the substrate of germination (Stendahl, 2005). In general, when the cotyledonary seeds reaches levels of 35% to 40%, the hydric potentials of environment and the seed get very next and with this, the absorption of water by seed stabilizes (Taylor, 1997), beginning a stationary phase, that is the phase II that occurs in function of balance between the osmotical potential and the pressure potential, where will occur the digestion and the active transport of substance of reserve, like protein, carbohydrates and lipids (Bewley, 1997). During the phase II, the metabolic process is activated, like related for seeds of *Brassica*

napus L. (Li et al., 2005), *B. oleracea L.* (Soeda et al., 2005) and *Raphanus sativus* L. (Berger et al., 1995). In this phase, despite of slow absorption of water, the embryonic axis still can not growth.

The phase III corresponds to the emission of primary roots and posterior cellular elongation (Stendahl, 2005). According Bewley and Black (1994), only viable and no-dormant seeds reaches this phase.

This standard three-phase of water absorption was observed in seeds of several species, like soybean (McDonald & Armstrong, 1992) and cotton (Prisco et al., 1992), being that the duration of each phase varies between the species and inside the same specie (Albuquerque et al., 2000). However, not always this standard three-phase can be characterized, because a big interval between the weighing can lost the inflexion points of imbibition curve that characterize each phase (Nóbrega, 1993).

During the imbibition of seeds occurs loss and gains of oxygen what defines the format of imbibition curve. The sensibility to the partial loss of oxygen varies between the species (Corbineau & Côme, 1995). In general, seeds of monocotyledons are less sensitive to the loss of oxygen than the dicotyledons species. Also seeds with high content of lipids are more sensitive to the loss of oxygen than those seeds with content of starch (Finch-Savage et al., 2005). This sensibility to the oxygen is related to the germination.

The consume of oxygen can vary between cultivars of same species, like observed to canola seeds (Barber et al., 1991), which suggests that genetic components influence in this variation.

The imbibition curve is an important technical procedure which aimed to improve the quality of seeds involving tests that evaluate the viability and vigor. According with Carvalho & Nakagawa (2000), the importance of determination of absorption water curve of one specie is related to the studies of impermeability of tegument, determination of duration of treatment with vegetables regulators, osmotic conditioning and pre-hydration.

Any critical decision related to these tests must be done while the seeds remain in the phase II (Bradford, 1995). Besides this, most of works realizes the construction of imbibition curve using different samples of seeds in each interval of time (Bittencourt et al., 2004; Bortolotto, 2007; Posse et al., 2001). Works that use the same sample of seeds (Justo et al., 2007) are scarce.

For oil radish, *Raphanus sativus*L. var. oleiferus Metzg., culture with big potential for utilization in production of biodiesel by having 30% to 40% of oil in seeds (Vieira, 2007), studies who aim to evaluate the behavior of seeds before the radicular protrusion are incipient.

Thus, the objective of this research was determinate the standard and the duration of water imbibition phases of oil radish seeds.

Material and Methods

The experiments were realized at Universidade Federal de Lavras, in Laboratory of Seeds Analysis, in Lavras MG. Were used lots of seeds of cultivars CATI AL-1000, from 2004 (lot 1) and IPR 116, from 2004 (lot 2) and 2005 (lot 3). The research was conducted in two experiments, being the experiment 1 realized with the same sample of seeds weighed at each interval of time, and the experiment 2 with different samples.

In both experiments, seeds were placed to soak in acrylic box, type gerbox, over moistened paper with distilled water 2,0 times the weight of the dry substrate, at 25°C, with constant light in Biochemical Oxygen Demand (B.O.D.) chamber.

During the evaluation, the seeds were removed from the gerbox, carefully dried with the assistance of towel paper and weighed in digital balance with precision of 0.0001g.

The seeds were weighed before the inception of imbibition and in intervals of time of 0; 4; and 15 minutes, 2; 4; 6; and 12 hours, being each interval with duration of 12 hours. The curve was accompanied for 7 days.

The increment of mass (I) was calculated over the time in function of initial seed mass (Bewley & Black, 1994); I (%)= [(Mt - Mi)/ Mi] x100, where: Mi = initial fresh mass of sample and Mt = mass of sample in time (t).

Tried to establish for each treatment one equation of 3rd degree that adjust to the standard three-phase of germination and delineate the beginning, the final and the duration of phases in the germination process. After the derivation of the 3rd degree equation was determinate the square root of derived equation of 2rd degree and the points of inflexion of curves, expressed in percentage of increment of mass per hours ($\%.h^{-1}$), considering the midpoint of each interval for each value of x.

The dates were submitted to analysis of variance and regression, with the aid of statistical program SISVAR® (Ferreira, 2000), with four replication of 50 seeds.

Results and Discussion

Experiment 1- For the cultivar CATI AL-1000, the duration of phase I was of 28,55 hours, with the end of phase II at 35,87 hours. For this cultivar, the phase II was very short. For the cultivar IPR 116, in both lots, was observed just the phases I and III, being that the lot from 2004 had the end of phase I with 18,05 hours and the lot from 2005 with 21,32 hours, starting at this point, the phase III. The absence of three-phase standard can be justified by having realized the weighing of same sample of seeds in all the intervals of time, what favored the removal of seed coat, resulting in the emission of radicle. In the Figure 1, was observed the results (in hours) of

initial, final and duration of phases of imbibition process of oil radish seeds when utilized the same sample of seeds for the evaluation of weighing.

For *B. oleraceae* L., McCormar et al. (1990) also observed a fast imbibition in the beginning of germinative process, being this fact associated to damage in the tegument of seeds. However, according Castro & Hilhorst (2004), the phase II is important, because it is in this phase that occurs synthesis and duplication of DNA and the beginning of reserves degradation and the cellular elongation.

For castor bean seeds, was not also observed the formation of phase II (Souza, 2007), even with the removal of caruncle (Oliveira et al., 2004).

Experiment 2- The three-phase standard was evident when was used different sample in the weighing to obtain the imbibition curve. The beginning of phase II in the process of imbibition for cultivar CATI AL-1000 occurs with 49,07 hours, for the IPR 116, lot from 2004 with 52,55 hours, and for the lot of 2005 with 58,92 hours from the beginning of imbibition. The end of phase II occurs with 116,93 hours for CATI AL-1000 cultivar, 152,78 hours for IPR 116, lot from 2004, and 139,74 hours for lot from 2005 (Figure 2). The phase III was achieved from the protrusion of radicle in seeds.

The phase III is characterized by radicle protrusion and per a resumption of water absorption by seeds. However, contrary to the phase I, this phase has active absorption of water in seeds (Black & Bewley, 1994).

The imbibition curves of aspargus, *Asparagus officinalis*, also presented the typical three-phase standard, existing fast hydration of seed, with protrusion of primary roots from 120 hours (5^o day) of imbibition (Bittencourt et al., 2004). Seeds of soybean (Braccini, 1998) and onion (Lopes et al., 1996) also have three-phase behavior for imbibition curve of seeds.

According with Wrasse (2006), the water and the oxygen that can pass through the tegument of seeds are availed in an efficient way for the metabolism of vigorous seeds. This metabolism requires amounts of water each time higher, giving greater speed to the hydration and levels more elevated in the first hours. Was observed in Figure 2, for all lots, a fast imbibition in the first hours.

Lopes (1996) verified that the duration of equilibrium phase or stabilization in the absorption of water (phase II) for onion seeds, occurs between 24 and 72 hours. Seeds of peas, beans and soybean, species that contains oil similar to oil radish, can remain in the phase II for a period eight times to ten times superior than the observed in the phase I, while the seeds of wheat and rice practically not presented the phase II, Copeland, 1976 (cited per Lopes, 1996).

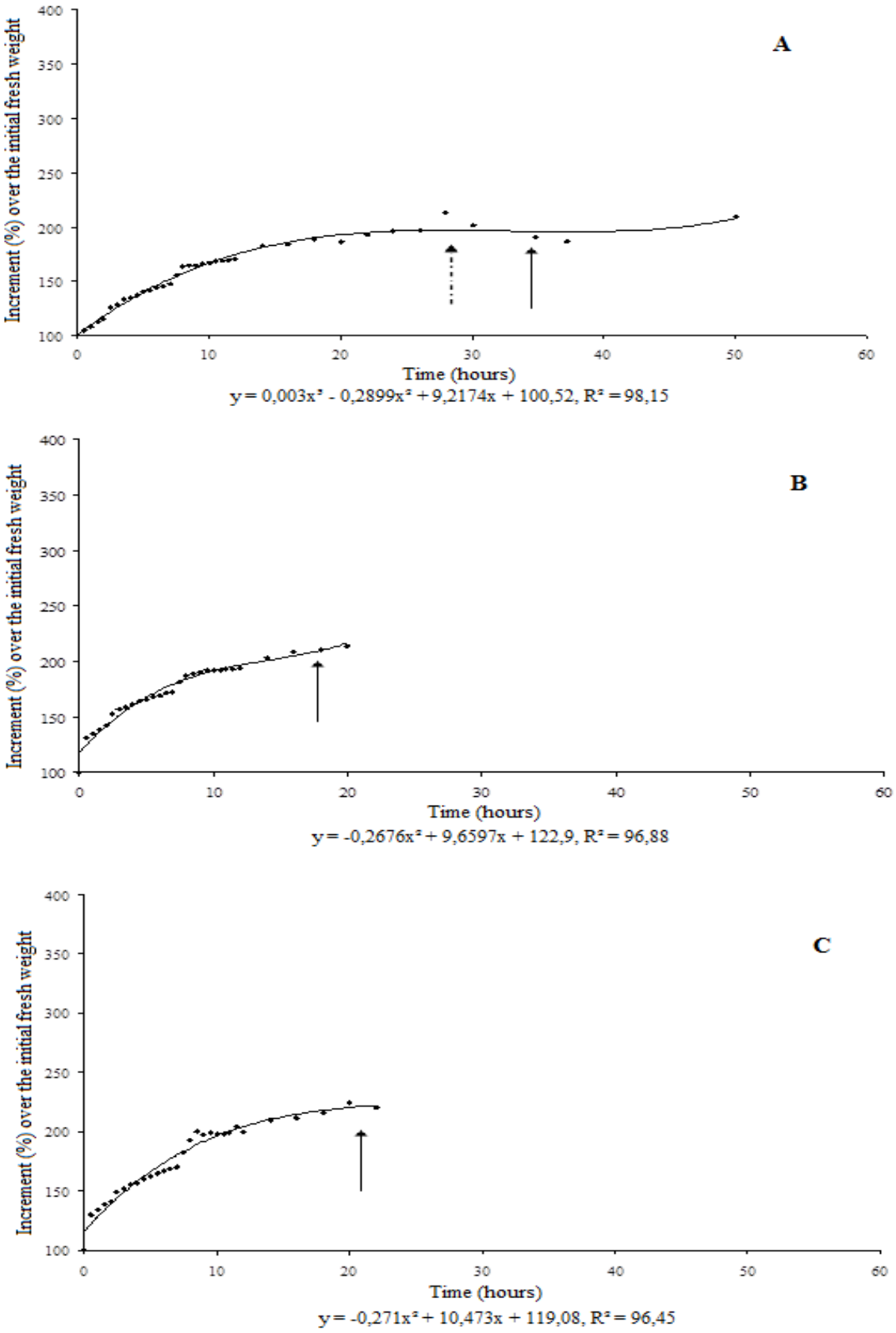

Figure 1.Imbibition curve of oil radish seeds cultivar CATI AL-1000, lot 2004 (A); IPR 116, lot 2004 (B) and IPR 116, lot 2005 (C), when the same sample of seeds was used in the weighing. Beginning of phase II (dashed arrow) and beginning of phase III (full arrow).

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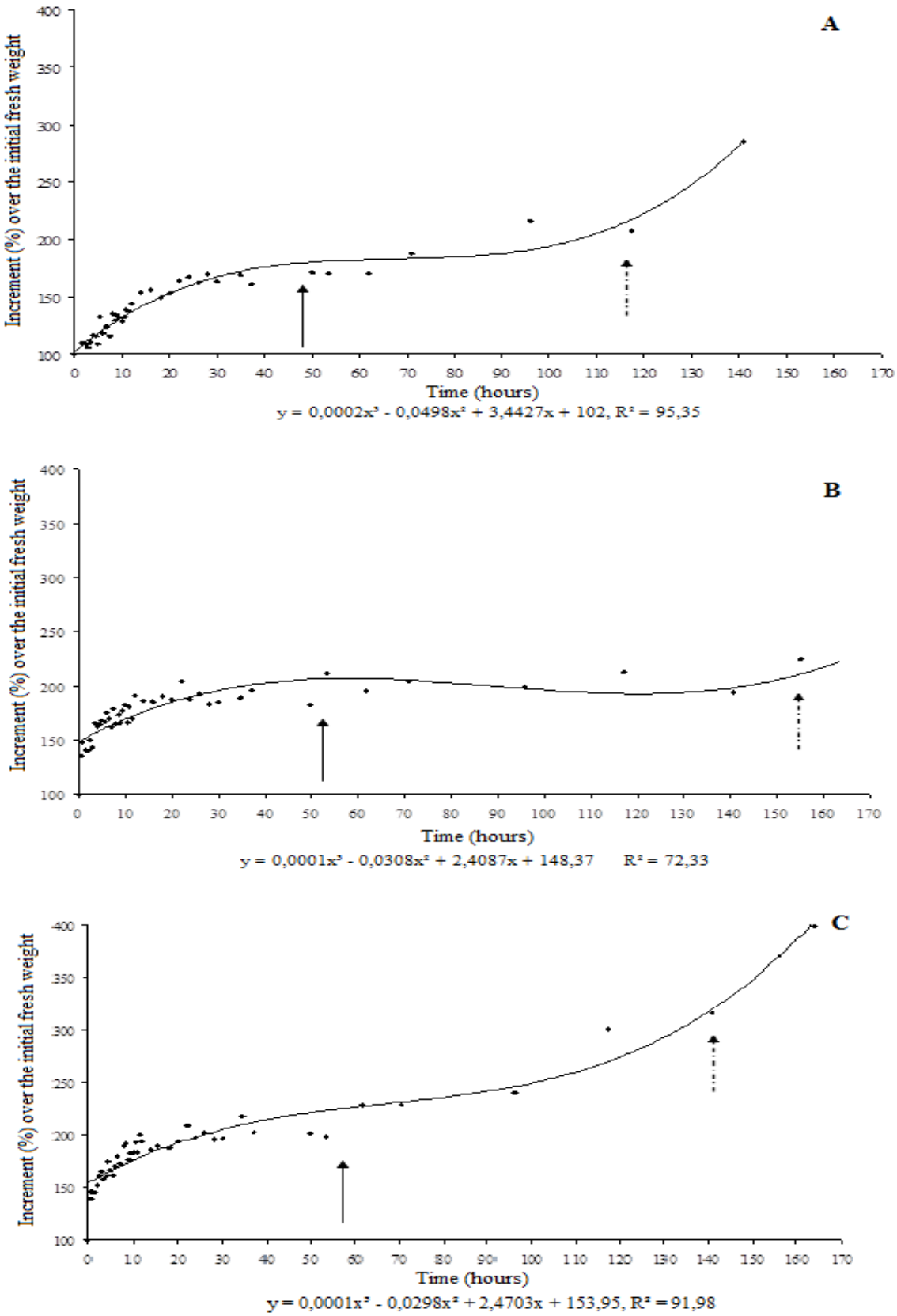

Figure 2.Imbibition curve of oil radish seeds cultivar CATI AL-1000, lot 2004 (A); IPR 116, lot 2004 (B) and IPR 116, lot 2005 (C), when different sample of seeds was used in the weighing. Begining of phase II (dashed arrow) and begining of radicle protrusion (full arrow).

Conclusion

With the same sample in the weighing, for the cultivar CATI AL-1000, the duration of phase I, is of 28,55 hours, with the end of phase II at 35,87 hours. For the cultivar IPR 116, the lot from 2004 is the end of phase I with 18,05 hours and the lot from 2005 with 21,32 hours, starting from this point, the phase III.

With different sample of seeds for the cultivar CATI AL-1000, the duration of phase I occurs with 49,07 hours, for the cultivar IPR 116, the lot from 2004 with 52,55 hours and for the lot from 2005 with 58,92 hours. The end of phase II occurs with 116,93 hours, 152,78 hours and 139,74 hours, respectively.

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