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The development of RBL-STEM learning materials to improve the students' combinatorial thinking skills in solving transgenic sugarcane confirmation problem using PCR technique

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Abstract

Combinatorial thinking skills are essential for integrating parameters such as annealing temperature, DNA template volume, and the number of cycles to optimize polymerase chain reaction (PCR) techniques. This study aims to develop research-based learning (RBL) integrated with science, technology, engineering, and mathematics (STEM) learning materials to enhance students' combinatorial thinking skills in confirming transgenic sugarcane using PCR. The development process followed the 4-D model, resulting in task plans (RTM), student worksheets (LKM), and student learning outcome tests (THBM). Validation was conducted by experts, while practicality and effectiveness were assessed through a small-scale implementation involving 25 students, utilizing pre- and post-tests, observations, and questionnaires. The learning materials were deemed valid (mean = 3.6), practical (mean = 3.8), and effective, as indicated by THBM (84%), activity scores (mean = 3.7), and positive responses (87%). Analysis of pre-test and post-test results demonstrated an increase in students' combinatorial thinking skills, with students categorized into low, medium, and high levels. The RBL-STEM learning materials effectively support the development of combinatorial thinking and problem-solving skills in molecular biotechnology. These materials present a promising approach to improving combinatorial thinking in biotechnology and can be adapted to other complex scientific topics that require analytical integration.

Keywords: Combinatorial thinking, PCR technique, RBL-STEM, Transgenic sugar cane.

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1. Introduction

As science and technology advance, students' skills in higher-order thinking are increasingly needed, including combinatorial thinking. This skill enables students to evaluate multiple solution pathways by considering alternative combinations and making logical connections between processes and outcomes [1, 2]. Core indicators of combinatorial thinking include investigation, pattern recognition, generalization, proof, and further exploration [3].

In biotechnology, to detect genetically modified organisms (GMOs), polymerase chain reaction (PCR) is a crucial method for amplifying DNA, including transgenic sugarcane with high sucrose content [4-6]. However, PCR is sensitive to parameters such as DNA template volume, annealing temperature, and number of cycles [7-9]. Inconsistent results, such as weak DNA bands and mismatches, can lead to false positives or negatives, requiring students to apply combinatorial thinking to address such issues [10-12].

In responding to these needs, alternative learning models, including project-based learning (PjBL), problem-based learning (PBL), and research-based learning (RBL), have been widely developed [13, 14]. Among these models, RBL is considered the most appropriate in the context of transgenic sugarcane confirmation because it emphasizes the activities of examining, investigating, and applying ideas in real situations [15]. RBL encourages students to resolve issues through authentic research activities in the curriculum [16]. In contrast, PBL emphasizes more on formulating pre-existing problems, while PjBL emphasizes more on creating project products [17, 18]. These characteristics make RBL suitable for application to improve combinatorial thinking skills because it encourages students to explore various possible solutions from different combinations of variables. In its application, the RBL model is often combined with a specific learning approach, and some studies recommend a STEM approach [19, 20].

STEM here means teaching that incorporates interdisciplinary approaches from the four domains, emphasizing authentic problem solving [21]. Previous findings reported that in biotechnology courses, STEM can create a more active and positive learning environment [22, 23]. STEM-based learning encourages hands-on inquiry [24] and focus on solving real problems [25, 26] which makes learning more in-depth and relevant for students.

The integration of RBL-STEM is a perfect fit because both share the same organizational emphasis, namely an emphasis on active student involvement in authentic problem-solving. This integration encourages students to engage in the research process and presents real-life phenomena in class [27]. RBL-STEM learning motivates students to do more than just comprehend the concepts of each field; it also encourages them to integrate cross-disciplinary knowledge to produce innovative solutions through the research process [16]. Empirical evidence shows that RBL-STEM can improve higher-order thinking skills, including combinatorial thinking skills [28-30]. This model promotes a research-oriented learning environment that engages students in collaborative inquiry and real-world problem-solving, building a link between theory and practice [1, 27, 30, 31].

Although relevant, studies focusing specifically on enhancing combinatorial thinking skills through biotechnology learning, especially in PCR-based transgenic confirmation, remain limited. Therefore, to ensure that research is meaningfully integrated into the learning process, it is important to design and implement effective RBL-STEM-based learning materials. These materials can guide students taking biotechnology courses in implementing optimal PCR techniques by considering all existing parameters. This study focuses on developing and evaluating learning materials based on RBL-STEM to improve students' combinatorial thinking skills in the context of transgenic sugarcane confirmation through PCR techniques. Furthermore, we examine the outcomes of reviewers' validation of the learning materials and the outcomes of their implementation for students taking biotechnology courses.

2. Research Method

This study employed a research and development (R&D) design with a mixed-method approach (qualitative and quantitative). The developed product consisted of RBL-STEM-based learning materials, including the student assignment plan (LKM), student worksheet (LKM), and learning outcome test (THBM), which was designed through the Define, Design, Develop, and Disseminate (4-D) stages, referring to Thiagarajan et al. [32]. The development procedure is illustrated in Figure 1.

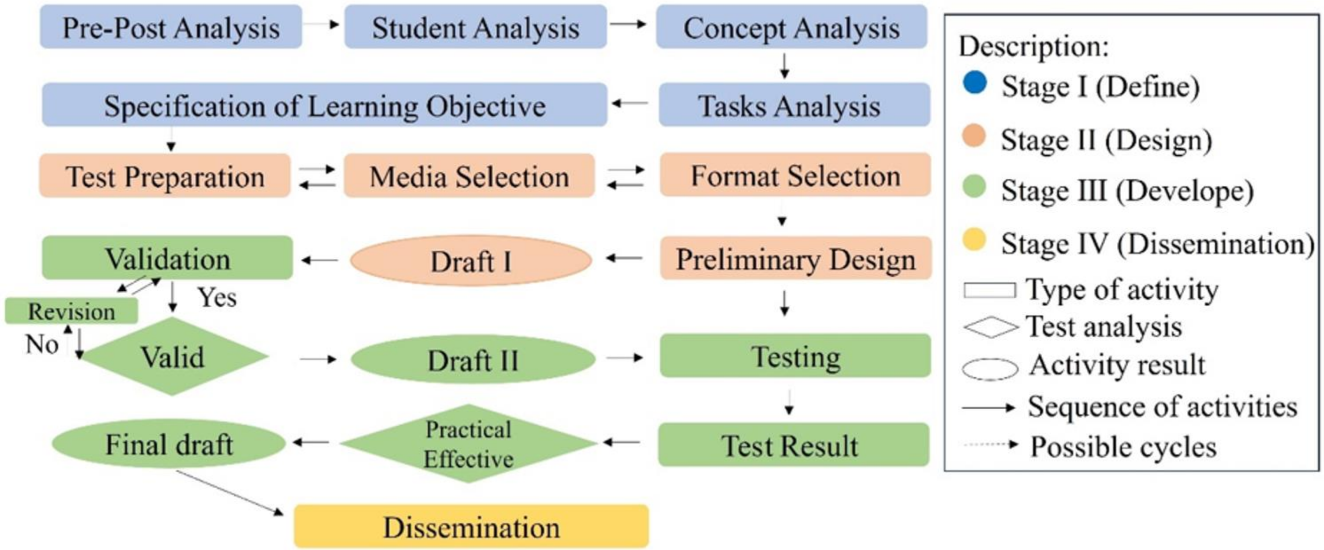

Figure 1.
4-D Model Design.

The Define stage aims to identify existing needs and challenges related to PCR technique instruction. This stage begins with a pre-post analysis to identify specific learning problems and needs related to PCR instruction. Furthermore, classroom observations are used to determine the characteristics and backgrounds of students who will be targeted for research and to analyze biotechnology learning at universities. Task analysis is carried out to consider the skills needed to master the PCR technique. Meanwhile, concept analysis is used to compile the main materials that students must master, and learning objectives are formulated to ensure that they are appropriate, measurable, and aligned with learning needs.

In the Design stage, a prototype draft (RTM, LKM, and THBM) and the instruments were constructed on the RBL-STEM. The content was intentionally structured to improve students' combinatorial thinking skills. In the Development stage, the learning materials were refined and improved based on expert validation and the results of limited trials. This stage involves producing learning materials that meet validity criteria (format, content, and language). The limited trial in this study was conducted in 2023 with 25 students of the Agrotechnology Study Program at the University of Jember who were enrolled in the Biotechnology course. Additionally, data collection and analysis were obtained from the results of semi-summative evaluations to assess the practicality and effectiveness of learning. Finally, the dissemination stage aims to distribute learning in other classes or through presentations at conferences or scientific journal publications.

Validity data analysis follows, while practicality and effectiveness follow Marsidi et al. [33]. The results of the pre-test and post-test trials were further analyzed using SPSS 24, which includes normality tests and paired sample t-tests. In addition, we also analyzed qualitatively using NVivo 15 to visualize the mindset of students who have different combinatorial thinking skills. The analysis carried out includes student phase portraits, adjacency matrices, topography matrices, word clouds, word trees, comparisons, and project maps.

3. Research Results

The development and testing of RBL-STEM learning materials in this study were analyzed both quantitatively and qualitatively. The findings include assessments of validity, practicality, effectiveness, and an in-depth understanding of students' combinatorial thinking processes. Visual representations such as phase portraits, Total Depth (TD), Relative Asymmetry (RA), and word cloud analysis were employed to observe how students learn and think when confronted with the problem of transgenic sugarcane confirmation using the PCR technique. These visual tools facilitated the interpretation of students' learning behaviors in solving PCR-based problems related to transgenic sugarcane confirmation.

3.1. Define

A needs analysis was conducted by reviewing literature, conducting interviews, and observing classrooms. The results revealed that students had difficulty managing complex PCR parameters. Additionally, students exhibited diverse levels of foundational knowledge, clearly highlighting the need for a learning approach that integrates theoretical concepts with hands-on laboratory practice. Based on the analysis, specific learning objectives were formulated by incorporating STEM aspects into the resolution of biotechnology-related problems, as shown in Figure 2.

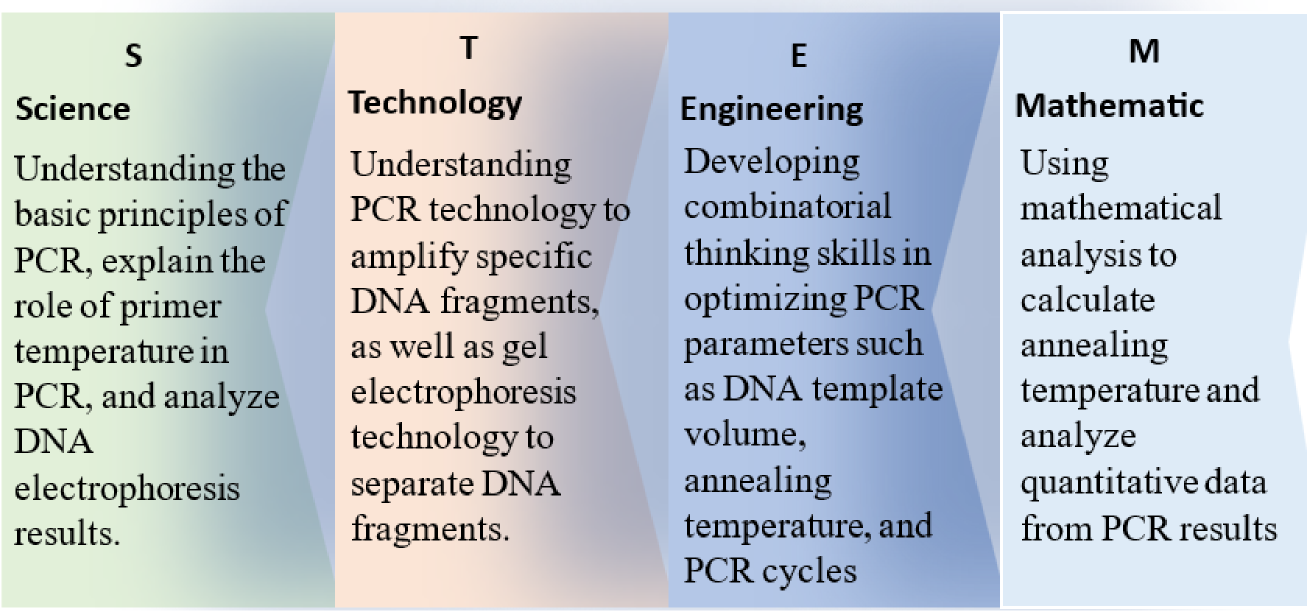

Figure 2.
STEM Aspects.

RBL-STEM learning materials offer an integrated and comprehensive approach. In the science aspect, students are guided to understand the basic principles and components involved in the PCR process. An understanding of PCR technology, which can amplify DNA, is also taught. Not only that, in the engineering aspect, students are encouraged to be able to optimize by considering all PCR parameters so that the PCR process can run optimally. Mathematical aspects are also included in learning, such as calculating the annealing temperature and calculating the number of PCR parameter combinations. The RBL-STEM syntax frame consists of six steps Figure 3.

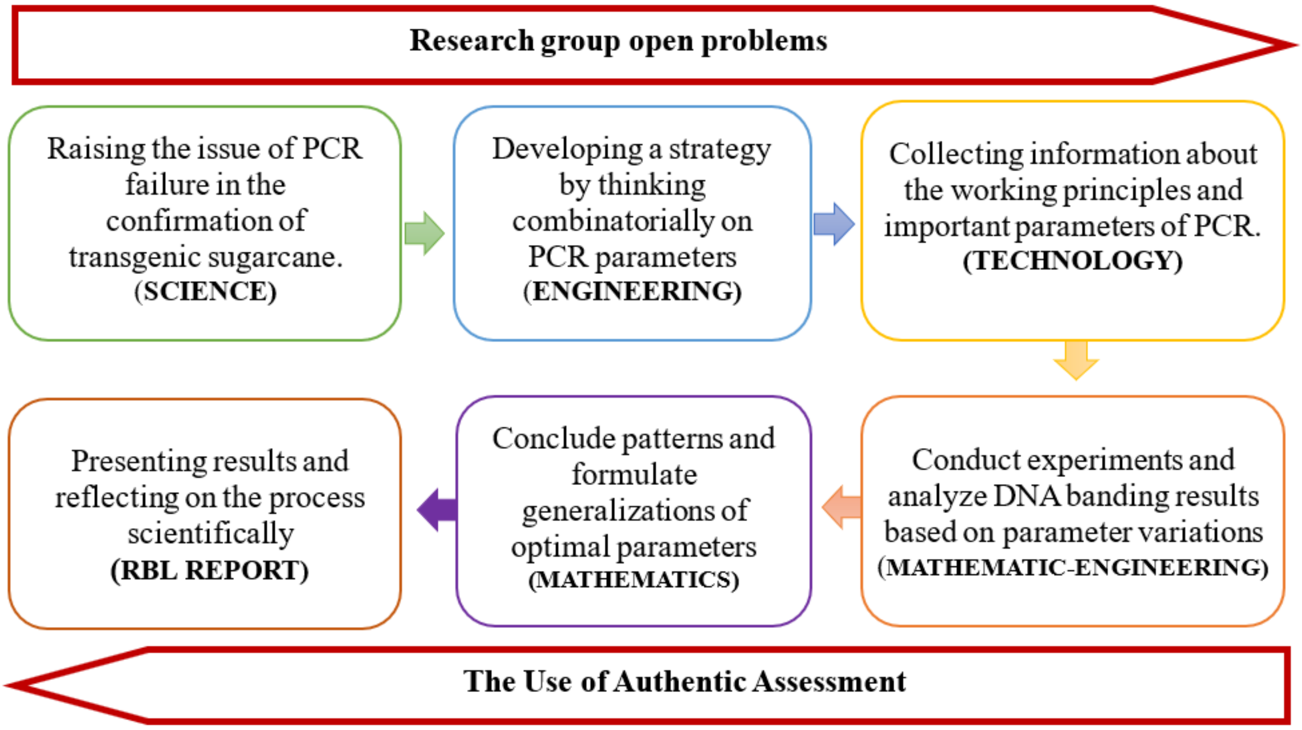

Figure 3.
RBL-STEM Syntax Framework.

In the first step of the process, students are presented with an open problem concerning PCR failure in confirming transgenic sugarcane. The types of PCR failures and their impact on the confirmation of transgenic sugarcane are examined at this stage to help students understand the fundamental concepts of real-world problems. Furthermore, in the second step, students explore strategies in the form of solutions by designing a combination of PCR parameters to optimize the confirmation process of transgenic sugarcane using PCR.

The third step emphasizes students collecting information that can support the strategies they have created through official protocols and scientific journals. Information such as DNA template volume, annealing temperature, and number of cycles is searched and discussed to obtain important points that can be used as references in conducting experiments. The fourth step focuses on experiments and analysis of results. Students are facilitated to conduct PCR and electrophoresis experiments to test the effectiveness of the combination of PCR parameters they have designed.

The fifth step involves the generalization of the process and findings, where students are asked to conclude the scientific patterns and principles of the entire experiment. Students are engaged in the discussion process to analyze the results of electrophoresis data obtained from all groups and compare the intensity of the DNA bands formed in each combination. The sixth step is the presentation and reflection stage. Students compile and present a scientific report. This presentation includes the design of the experiment, the results of the analysis, the generalization of the group, as well as self-reflection.

3.2. Design

In the Design stage, the learning materials developed include RTM, LKM, and THBM. These materials are designed to facilitate students' active involvement in exploring concepts through RBL-STEM-based laboratory experiments. Additionally, development was carried out to compile several research instruments, such as validation sheets, activity observation sheets (for lecturers and students), and student response questionnaires. The prototype workflow was presented in Figure 4.

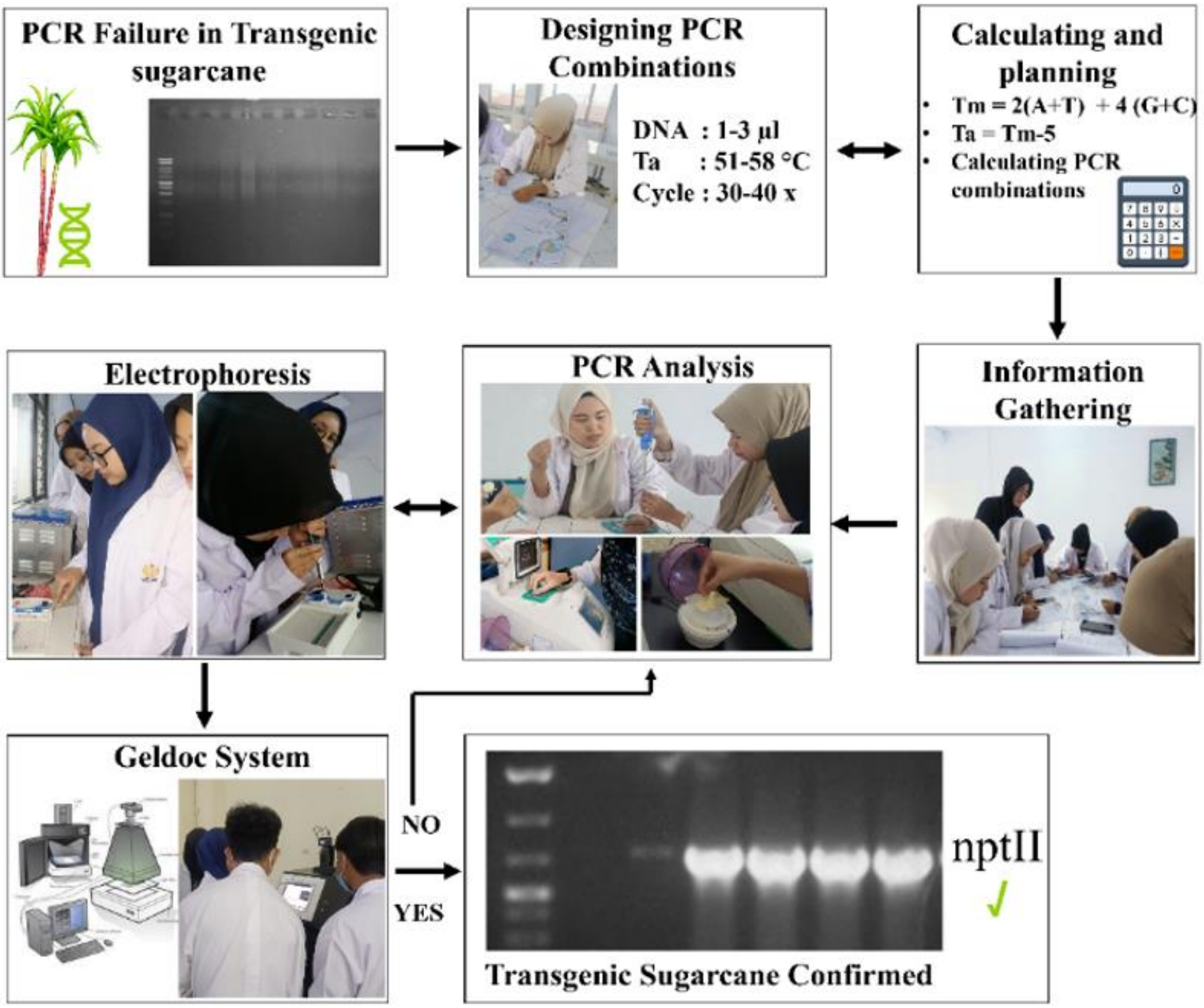

Figure 4.
Prototype Workflow for PCR-Based Confirmation of Transgenic Sugarcane.

Figure 5 shows the workflow steps in the process of transgenic sugarcane confirmation using PCR analysis. The process includes PCR combination design, Tm calculation, information collection, PCR amplification, electrophoresis, and gel documentation. Successful amplification is indicated by the presence of nptII bands. In the workflow, directional arrows (→) indicate sequential steps, and bidirectional arrows (↔) indicate iterative processes that require adjustment or re-evaluation when the results are not in accordance. This combination ensures procedural flow and feedback-based problem-solving to improve accuracy.

3.3. Develop

Three validators verified the learning materials, including expert lecturers in education, biotechnology, and language, and assessed their practicality and effectiveness based on small-scale implementation with students. The following results were obtained:

3.3.1. Validity Testing

The validity criteria of the learning materials were assessed using a Likert scale of 1-4, and the results are summarized in Table 1.

Table 1.
Recapitulation of Learning Materials Validation Results.

Materials				
Aspect	**Score**	**RTM**	**LKM**	**THBM**
Format	Average score	3.0	3.7	3.7
	Average percentage	75%	93%	93%
Content	Average score	3.3	3.5	3.7
	Average percentage	83%	88%	93%
Language	Average score	3.7	3.9	4
	Average percentage	93%	98%	100%
Overall aspect average score		3.3	3.7	3.8
Overall aspect average percentage		83%	93%	95%
Conclusion		Valid		

The validation results show that the overall average value was 3.6, which is in the range $3.25 \leq Va < 4.00$ and falls into the “valid” category. This finding indicates that, in terms of content, format, and linguistic appropriateness, especially in the THBM component, it has met the eligibility criteria.

3.3.2. Practicality Test

Three observers were involved in the learning process. The practicality test was assessed by analyzing the viability of classroom instruction based on the activities of the lecture. The outcome of the observation is shown in Table 2.

Table 2.
Summary of Learning Process Observations.

No	**Assessed Aspect**	**Average**	**Percentage**
I	Syntax	4	100%
II	Social System	3.7	92%
III	Principles of Reaction and Management	3.7	92%
Overall average score		3.8	94%

The observation results yielded an average score of 3.8, equivalent to 94%, which lies within the interval $90\% \leq SR \leq 100\%$, indicating that the learning implementation is categorized as "very practical".

3.3.3. Effectiveness Test

The effectiveness test assessed the extent to which the learning materials improved students' combinatorial thinking skills in addressing the confirmation of transgenic sugarcane. The results of this evaluation are presented as follows:

3.3.3.1. Analysis of THBM

About 21 out of 25 students scored above the minimum passing score of 75, which is 84%. These results show that the learning material effectively helps students achieve classical completeness. It also indicates an improvement in students' understanding and use of combinatorial thinking skills.

3.3.3.2. Analysis of Students' Activities

The recapitulation of the student activities during the learning process is presented in Table 3. Based on the results of the recapitulation, the percentage obtained with an average score of 3.7 and a percentage of 92.5% indicates that the students fall into the "very active" category.

Table 3.
Summary of Student Activity Observation.

Indicator	Mean score	Percentage
Opening Session	4	100%
Core Learning Process	3.60	90%
Lesson Conclusion	3.5	87.5%
Overall average	3.7	92.5%

3.3.3.3. Analysis of the Questionnaire

The response questionnaire was distributed to 25 students to assess their level of acceptance of the learning materials. The questionnaire included "Yes" and "No" answers, where students evaluated whether the learning process met the indicators based on their experiences during the learning sessions.

Table 4.
Results of Data Recapitulation of Student Response Questionnaire Results.

No	Aspect	Statement number	Yes	No	Yes (%)	No (%)
1	Learning components	1-4	22.25	2.75	89	11
2	Content novelty	5-8	23.25	1.75	93	7
3	Engagement	9	19	6	76	24
4	Language clarity	10-11	21.5	3.5	86	14
5	Comprehension of tasks	12-13	22.5	2.5	90	10
6	Visual design	14-15	22	3	88	12
7	Group collaboration	16	22	3	88	12
	Average		21.79	3.21	87	13

The student response questionnaire regarding the implementation of RBL-STEM resulted in an average of 87% of students responding positively ("yes"), while 13% responded negatively ("no"). This indicates that the majority of students are satisfied with and enjoy using the RBL-STEM learning materials. Therefore, according to the established criteria, these learning materials are effective in supporting the learning process.

After development, the learning was implemented on a larger scale. The results of the pre-test and post-test conducted during this phase are shown in the figure.

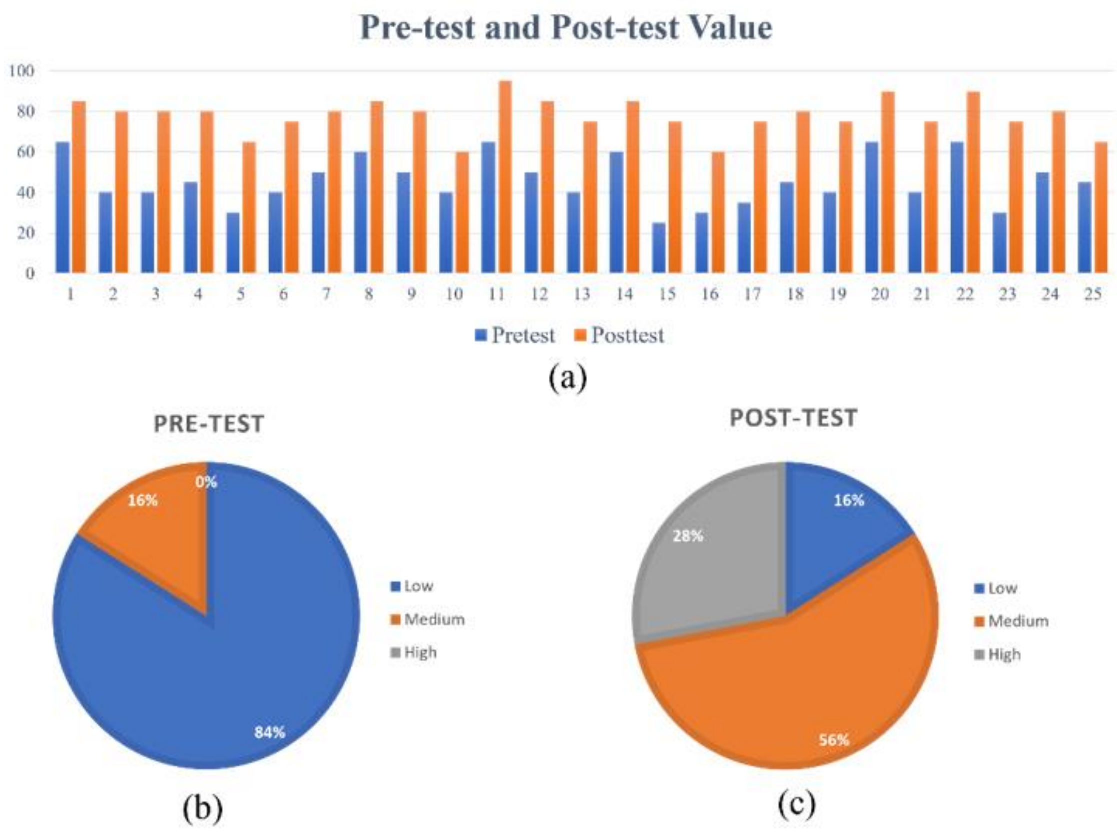

Figure 5.
Distribution of Students' Combinatorial Thinking Skills: a. Pre-test and Post-test Results of Each Student, b. Pre-test Percentage, c. Post-test Percentage.

The pre-test findings showed that students' combinatorial thinking skills initially consisted of two levels, namely low (84%) and medium (16%). The post-test results show an improvement in the distribution of skills across the three levels: 28% at the high level, 56% at the medium level, and 16% at the low level. Additionally, a normality test was conducted using SPSS 24 software as a precondition for the paired sample t-test, and the results are shown in Table 5.

Table 5.
Normality Test Result.

Test of Normality						
	Kolmogorov-Sminov[a]			**Shapiro-Wilk**		
	Statistic	**df**	**Sig.**	**Statistic**	**df**	**Sig.**
Pre-test	0.165	25	0.078	0.923	25	0.060
Post-test	0.206	25	0.008	0.933	25	0.103

Note: a. Liliefors Significance Corrections.

The results of the Shapiro-Wilk normality test show that the p-values are 0.060 and 0.103 ($p \geq 0.05$), indicating that the data are normally distributed. Further analysis to examine the impact of learning materials was conducted using a paired sample test Table 6.

Table 6.
Paired Sample T-Test Result.

Paired Samples Correlations				
		N	**Correlation**	**Sig.**
Pair 1	Pre-test & Post-test	25	0.774	0.000

The p-value of 0.00 ($p \leq 0.05$) from the results of the paired sample t-test revealed that there was a statistically significant difference between the pre-test and post-test scores. Therefore, it can be concluded that the application of learning materials using the RBL-STEM learning approach has significantly improved students' skills in identifying transgenic sugarcane through the PCR technique. This study developed context-specific indicators to assess students' combinatorial thinking skills in solving transgenic sugarcane confirmation using PCR techniques. These indicators are outlined in Table 7.

Table 7.
Combinatorial Thinking Skills in STEM Activities

E. 1 Indicator	E. 2 Sub-Indicator
A. Identifying several cases in the confirmation of transgenic sugarcane through the PCR technique	A. 1 Identifying factors causing PCR results to fail A. 2 Explaining the impact of PCR failure in the process of confirming transgenic sugarcane
B. Recognizing patterns from all cases	B. 1 Determining DNA volume B. 2 Calculating annealing temperature based on primer sequences B. 3 Finding the relationship between DNA volume, annealing temperature, and number of cycles on the intensity of the PCR DNA band
C. Generalizing all cases	C. 1 Creating a PCR parameter combination scheme and predicting results C. 2 Calculating all combinations
D. Proving systematically	D. 1 Analyzing the strengths and weaknesses of pattern-solving D. 2 Testing the validity of the optimal PCR parameter combination based on experimental results and scientific literature
E. Considering other combinatorial problems	E.1 Calculating all combinations, identifying other parameters outside of DNA volume, annealing temperature, and number of cycles that can affect the success of PCR.

To further substantiate the findings, a phase portrait analysis was conducted to visualize students' cognitive pathways during the problem-solving process. The phase portrait of Student 1 (S1), categorized as having low-level combinatorial thinking skills, is shown in Figure 6.

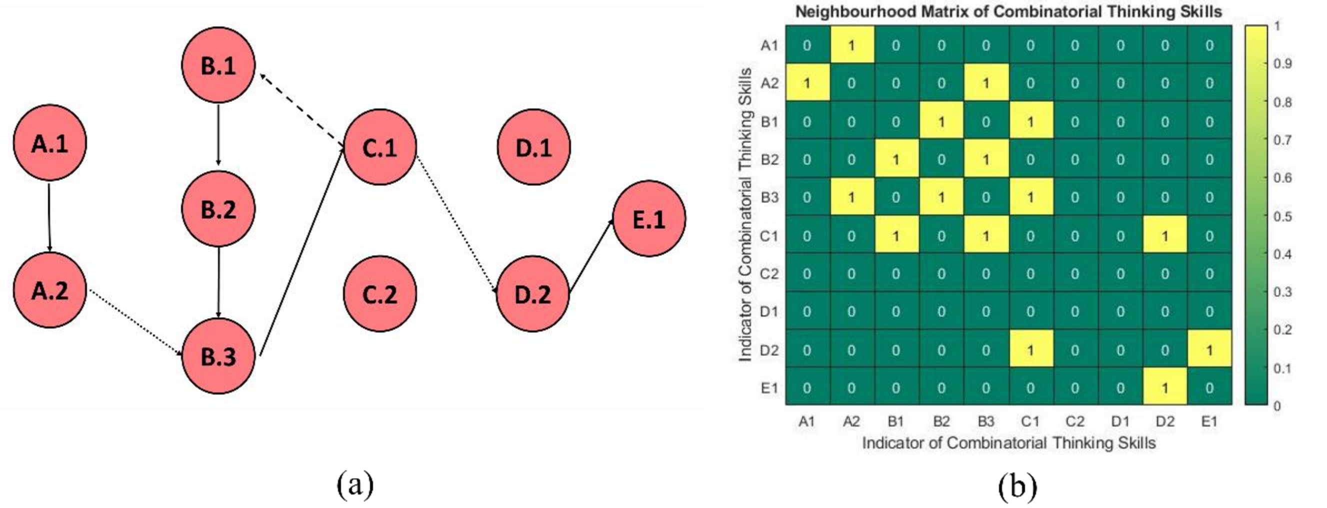

Figure 6.
Phase Portrait of S1: a. Graph representation, b. Adjacency matrix

Student 1 (S1) worked on the test starting from identifying several cases (A.1 and A.2), but his reasoning was inconsistent; instead of proceeding to B.1, the student immediately jumped to B.3 and C.1, even though he eventually reviewed indicators B.1 and B.2. Key indicators such as C.2 and D.1 were left out, and the process ended at E.1. This pattern indicates limited systematic reasoning and shallow integration of PCR parameter combinations. We used a number of flow metrics, including Total Depth (TD), Mean Depth (MD), Relative Asymmetry (RA), and Relative RA (RRA), to examine the topological features of students' thought routes to better comprehend their reasoning structure. These indicators help visualize the degree of connectivity, depth, and symmetry across each sub-indicator in the students' combinatorial reasoning. The topological metrics of S1 are summarized in Table 8.

Table 8.
TD, MD, RA, and RRA Values of Student 1's.

Sub-indicator	A.1	A.2	B.1	B.2	B.3	C.1	D.2	E.1
TD	26	19	25	25	14	14	19	26
MD	3.72	3.72	3.57	3.57	2	2	2.72	3.72
RA	0.91	0.57	0.86	0.86	0.33	0.33	0.57	0.91
RRA	2.49	1.57	2.36	2.36	0.92	0.92	1.57	2.49

The finding reveals that sub-indicators A.1 and E.1 have the highest TD values, suggesting that students frequently revisit these stages throughout their reasoning process. Levels are comparatively inefficient. In contrast, lower RA and RRA values suggest that students advanced through these stages more quickly. These findings show that during the RBL-STEM learning, B.3 and C.1 may serve as strategic checkpoints to help students build a more efficient thought process.

Student 2 (S2) was categorized as having medium combinatorial thinking skills, demonstrated a more structured reasoning pattern compared to the low-level skill, as shown in Figure 7.

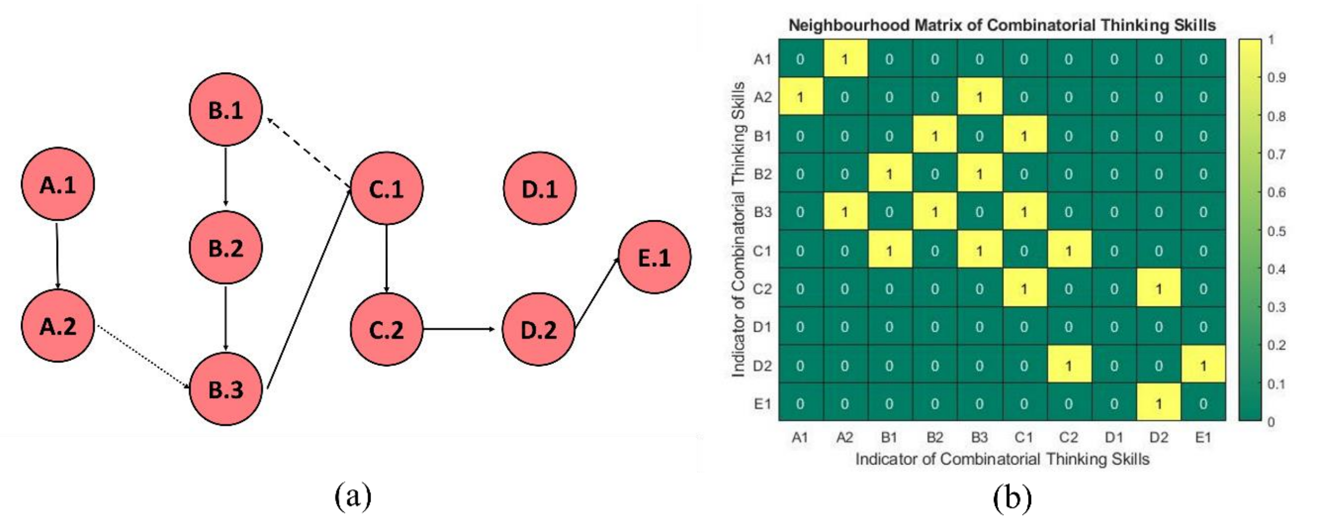

Figure 7.
Phase Portrait of S2: a. Graph representation, b. Adjacency matrix.

Student 2 can be seen to pass most of the indicators, except indicator D.1. This shows that the student has comprehensive and strategic reasoning, although there is a small gap in the evaluation. The overall reasoning remains comprehensive and strategic. Sub-indicators B2 and D3 had the highest TD. Table 9, showing frequent use, while lower RA values in B3 and C1 (RA=1.0 and 0.9) indicated more efficient transitions in those steps.

Table 9.
TD, MD, RA, and RRA Values of Student 2.

Sub-indicator	**A.1**	**A.2**	**B.1**	**B.2**	**B.3**	**C.1**	**C.2**	**D.2**	**E.1**
TD	32	24	29	35	18	17	21	27	35
MD	4.0	3.0	3.63	4.38	2.25	2.13	2.63	3.38	4.38
RA	0.86	0.57	0.75	0.96	0.35	0.32	0.46	0.68	0.96
RRA	2.4	1.6	2.1	2.7	1.0	0.9	1.3	1.9	2.7

The phase portrait of student 3 (S3) was shown in Fig. 8 to further demonstrate the reasoning trajectory of a student with advanced combinatorial thinking skills.

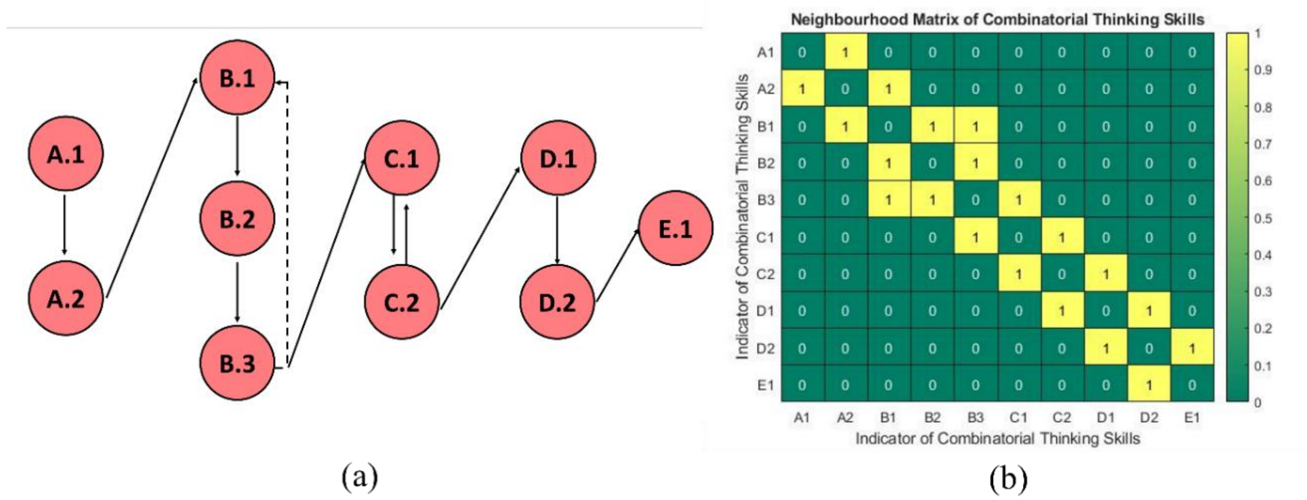

Figure 8.
Phase Portrait of S3: a. Graph representation, b. Adjacency matrix.

As shown in Figure 8, student 3, categorized as having high combinatorial thinking skills, demonstrated a more systematic and comprehensive process, completing all sub-indicators without omission. A more detailed quantitative analysis of these students' reasoning path was summarized in Table 10. The highest TD values were found at A1 and E1 (TD=45), showing full pathway transversal. Low RA values at B.3 and C.1 (RA=4) reflect efficient transitions, confirming strong integration and deep combinatorial reasoning throughout the RBL-STEM learning process.

Table 10.
TD, MD, RA, and RRA Values of Student 3's.

Sub-indicator	**A.1**	**A.2**	**B.1**	**B.2**	**B.3**	**C.1**	**C.2**	**D.1**	**D.2**	**E.1**
TD	45	37	31	27	25	25	27	31	37	45
MD	5	4.11	3.44	3	2.78	2.78	3	3.44	4.11	5
RA	1	0.77	0.61	0.5	0.44	0.44	0.5	0.61	0.78	1
RRA	2.85	2.22	1.74	1.43	1.27	1.27	1.43	1.74	2.21	2.85

NVivo software was used to code data to strengthen the qualitative analysis. The data obtained from students (S1, S2, and S3) were analyzed using NVivo, and a word cloud was presented in Figure 9.

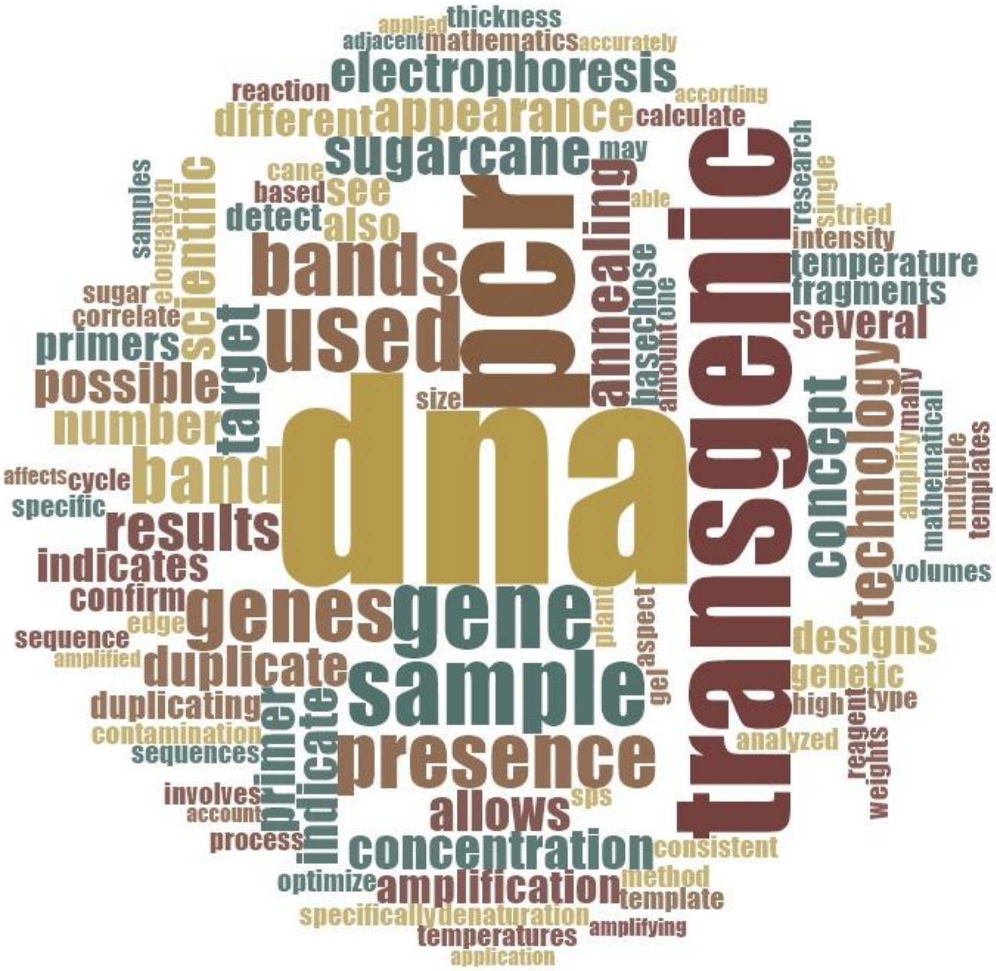

Figure 9.
Word Cloud from NVivo Analysis.

NVivo analysis was used to visualize the word frequencies from the student interviews (Fig.9). Larger words such as "DNA," "specific," and "PCR" were the most frequently occurring words, indicating that the learning process focused heavily on GMO confirmation using PCR. Other words, like "combination," "primer," and "cycle," reflected students' involvement in the experimental procedure, highlighting students' combinatorial thinking. These results are in line with the goal of RBL-STEM to directly integrate theory with practice. In addition to strengthening our qualitative analysis, we used query analysis to show the words that frequently appear together with DNA, and the results are shown in Figure 10.

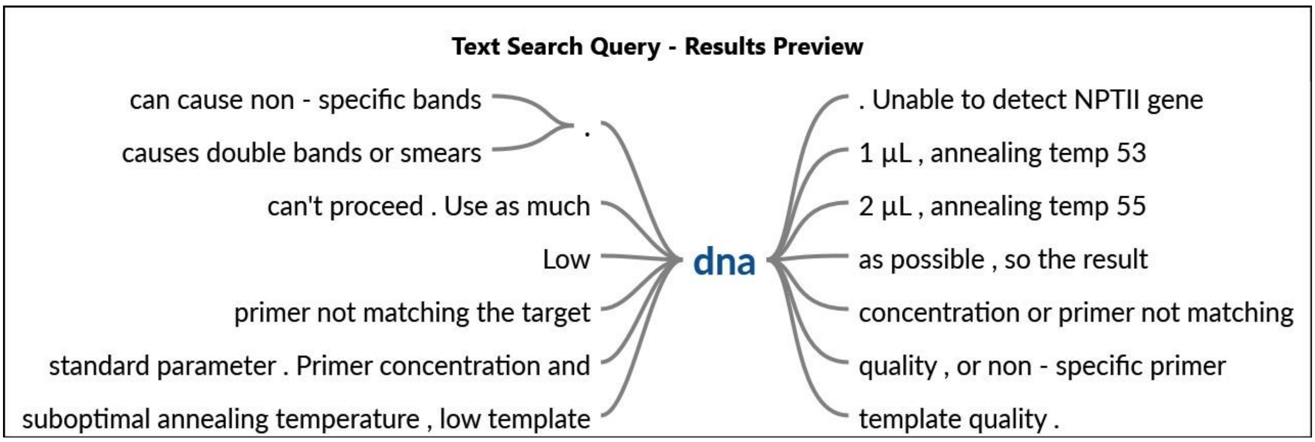

Figure 10.
Word Tree of The Word "DNA".

The DNA keyword results revealed several technical challenges experienced by students when confirming transgenic sugarcane, such as double non-specific bands or smears, and the inability to detect the NPTII gene. Other terms also appeared, such as DNA volume (1-2) and annealing temperature (53-55), indicating active student involvement in testing combinations of PCR parameters. This finding indicates students' combinatorial skills in understanding DNA quality from

PCR results, and this supports the effectiveness of RBL-STEM in the learning process. We conducted an NVivo analysis using the comparison feature to assess the differences in combinatorial thinking skills among students from different categories, which are presented in Figure 11.

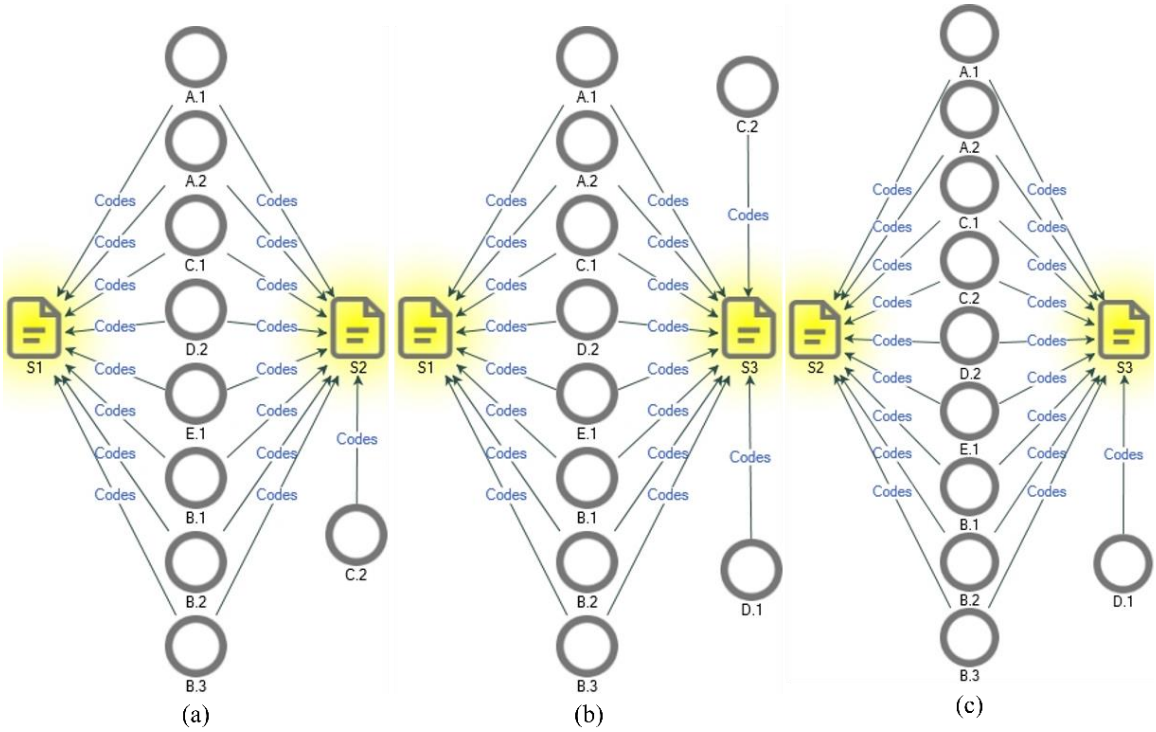

Figure 11.
Comparison of Students': (a) S1 and S2; (b) S2 with S3; (c) S1 and S3.

As shown in Figure 12, the differences in the level of combinatorial thinking skill indicators among the three students with varying skill categories are notable. The comparison between students 1 and S2 (Fig. 10a) reveals several similarities in their indicators; however, S2 demonstrates a broader range (indicator C.2), which S1 does not exhibit. Meanwhile, the comparison between S2 and S3 (Fig. 10b) shows an even wider range of indicators, with S3 outperforming S2 by incorporating indicators C2 and D.1. The comparison between S1 and S3 (Fig. 10c) highlights that S1 meets only 8 indicators, while S3 meets 10. This suggests that S3 is capable of identifying, analyzing, and addressing more aspects of the transgenic sugarcane confirmation process through PCR techniques. In contrast, S1's understanding is more limited, indicating that improvement is needed in several areas to achieve enhanced combinatorial thinking skills. A project map through NVivo analysis was conducted to map student involvement (S1, S2, S3) in the combinatorial thinking sub-indicators.

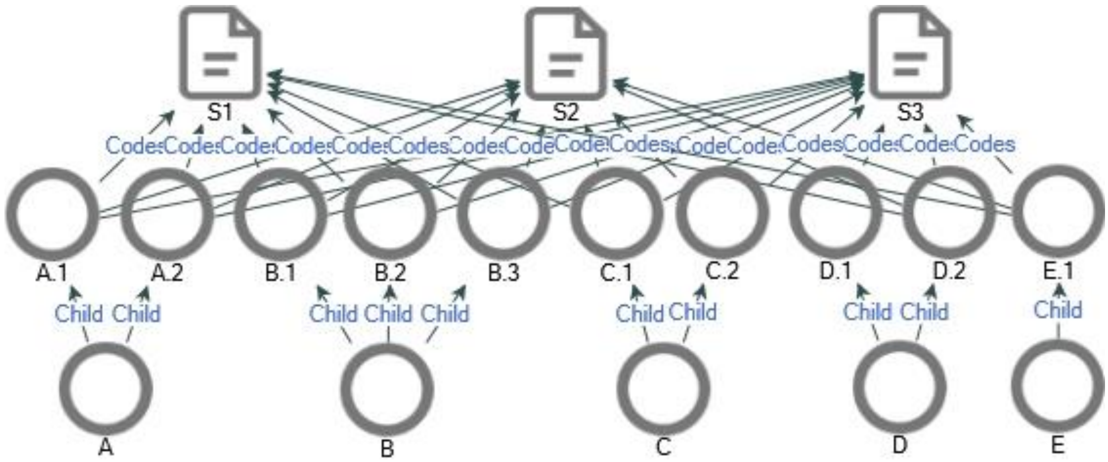

Figure 12.
Project Map of Student Combinatorial Thinking Skills.

The large circle on the project map shows the main indicator, and the child shows the sub-indicator. The project map results reveal that S1 only reaches some indicators, S2 is broader, while S3 can reach all indicators. This visualization supports the findings of the previous phase portrait analysis and strengthens the differences in combinatorial thinking skills between students, with S3 showing the most complete and systematic structure.

4. Discussion

This study successfully developed RBL-STEM-based learning materials that improved students' combinatorial thinking skills in solving problems related to transgenic sugarcane confirmation through the PCR technique. The materials strengthen conceptual understanding while promoting higher-order thinking, particularly in data analysis and synthesis within an authentic laboratory experiment. The findings support previous research on RBL-STEM learning materials using the irregular reflexive k-labeling [33]. Although differing in disciplinary focus, this study equally emphasizes the importance of cross-field integration, such as biology, mathematics, and technology, to solve complex and authentic problems. These findings demonstrate the flexibility of the RBL-STEM learning materials in adapting to molecular biotechnology contexts, which previously centered more on mathematics and technology [1, 14, 15, 31].

The field implementation revealed that the developed learning materials met high-quality standards in terms of validity, practicality, and effectiveness, especially in enhancing students' combinatorial thinking skills. These results align with previous studies claiming that the RBL-STEM model is more effective in developing combinatorial skills than conventional learning methods [34]. Through engagement in experimental activities and data interpretation, students applied theoretical knowledge to real-world challenges, an opportunity rarely afforded by conventional methods. Moreover, this study supports the development of important soft skills such as creativity, meta-literacy, and computational thinking [27, 31, 35].

This study expands the application of RBL-STEM learning into biotechnology education by guiding students in tackling combinatorial challenges in PCR-based transgenic confirmation. This demonstrates great potential in connecting theoretical concepts and practical applications across disciplines. However, the scope is limited to a specific technique (PCR). Future investigations should explore how combinatorial thinking can be cultivated in a broader context, such as bioinformatics, gene editing, or systems biology, to further validate and enrich the applicability of the RBL-STEM approach.

5. Conclusion

The RBL-STEM learning material developed has met the valid criteria as shown by the results of the expert lecturer validator, with an average value of 3.6, considered practical from the results of observations of lecturer activities, with an average percentage of 94%. These learning materials are also categorized as effective, as shown by 84% of students achieving the minimum standard score, 92.5% of students being actively involved, and 87% receiving positive responses. The pre-test and post-test results showed an increase in the scores of each student. Phase portrait analysis shows the flow of students' thinking supported by Total Depth, Relative Asymmetry, and word cloud data, indicating strong conceptual involvement. Thus, RBL-STEM is proven to be effective in improving students' combinatorial thinking skills in PCR confirmation of transgenic sugarcane.

References

[1] A. I. Kristiana, Z. R. Ridlo, R. M. Prihandini, and R. Adawiyah, "The research based learning--STEM learning activities: The use of r-dynamic coloring to improve the students metaliteracy in solving a tessellation decoration problem," *European Journal of Education and Pedagogy,* vol. 3, no. 4, pp. 52–60, 2022. https://doi.org/10.24018/ejedu.2022.3.4.381

[2] E. D. Putra, A. I. Kristiana, and A. Suwito, "Development of combinatorial thinking ability questions for students," *International Journal of Current Science Research and Review,* vol. 7, no. 9, pp. 7398–7404, 2024. https://doi.org/10.47191/ijcsrr/V7-i9-58

[3] J. Dafik, "On improving the students' combinatorial thinking skill in solving rainbow antimagic colouring problem on cryptography for e-commerce security systems under the implementation of research-based learning with STEM approach," *Innovare Journal of Education,* vol. 10, no. 5, pp. 21-30, 2022. https://doi.org/10.22159/ijoe.2022v10i5.45596

[4] B. Sugiharto, Z. R. A. Maulidia, T. Handoyo, and A. U. K. Maulidiya, "Transformation of the sosps1 gene using the osadh enhancer and embryogenic somatic callus explants in sugarcane plants," *Agriprima: Journal of Applied Agricultural Sciences,* vol. 6, no. 2, pp. 98-110, 2022. https://doi.org/10.25047/agriprima.v6i2.477

[5] R. M. Anur, N. Mufithah, W. D. Sawitri, H. Sakakibara, and B. Sugiharto, "Overexpression of sucrose phosphate synthase enhanced sucrose content and biomass production in transgenic sugarcane," *Plants,* vol. 9, no. 2, p. 200doi: https://doi.org/10.3390/plants9020200.

[6] P. Safaei, E. M. Aghaee, G. J. Khaniki, S. A. K. Afshari, and S. Rezaie, "A simple and accurate PCR method for detection of genetically modified rice," *Journal of Environmental Health Science and Engineering,* vol. 17, no. 2, pp. 847-851, 2019. https://doi.org/10.1007/s40201-019-00401-x

[7] I. P. Widyanto and E. T. Wahyuni, "Implementation of learning planning," *Satya Sastraharing,* vol. 4, no. 2, pp. 16–35, 2020.

[8] H. Zhang *et al.*, "Revealing the secrets of PCR," *Sensors and Actuators B: Chemical,* vol. 298, p. 126924, 2019. https://doi.org/10.1016/j.snb.2019.126924

[9] M. Banasik, A. Stanisławska-Sachadyn, and P. Sachadyn, "A simple modification of PCR thermal profile applied to evade persisting contamination," *Journal of Applied Genetics,* vol. 57, no. 3, pp. 409-415, 2016. https://doi.org/10.1007/s13353-015-0336-z

[10] D. J. Bacich, K. M. Sobek, J. L. Cummings, A. A. Atwood, and D. S. O'Keefe, "False negative results from using common PCR reagents," *BMC Research Notes,* vol. 4, no. 1, p. 457, 2011. https://doi.org/10.1186/1756-0500-4-457

[11] A. U. K. Maulidiya, B. Sugiharto, J. Waluyo, and I. Dafik, "RBL-STEM learning activities: Analysis of transgenic sugarcane development using artificial neural networks in improving students' combinatorial thinking skills," in *Proceedings of Atlantis Press International BV (Vol. 1). Atlantis Press.*, 2023, doi: https://doi.org/10.2991/978-94-6463-174-6_17.

[12] C. Winston, L. Organick, D. Ward, L. Ceze, K. Strauss, and Y. J. Chen, "Combinatorial PCR method for efficient, selective oligo retrieval from complex oligo pools," *ACS Synthetic Biology,* 2021. https://doi.org/10.1021/acssynbio.1c00482

[13] L. Zhang and Y. Ma, "A study of the impact of project-based learning on student learning effects: A meta-analysis study," *Frontiers in Psychology*, vol. 14, p. 1202728, 2023. https://doi.org/10.3389/fpsyg.2023.1202728
[14] R. Izza, A. I. Kristiana, and I. L. Mursyidah, "The development of RBL-STEM learning materials to improve students' combinatorial thinking skills in solving local (a, d)-edge antimagic coloring problems for line motif batik design," *European Journal of Education and Pedagogy*, vol. 4, no. 1, pp. 145-153, 2023. https://doi.org/10.24018/ejedu.2023.4.1.571
[15] Sumarno, Gimin, E. Noviana, Dafik, Z. R. Ridlo, and I. L. Mursyidah, "The analysis of the RBL-STEM application in improving student financial literacy in controlling consumptive behavior," *Heliyon*, vol. 10, no. 12, p. e32382, 2024. https://doi.org/10.1016/j.heliyon.2024.e32382
[16] F. Espinoza-Figueroa, D. Vanneste, B. Alvarado-Vanegas, K. Farfán-Pacheco, and S. Rodriguez-Giron, "Research-based learning (RBL): Added-value in tourism education," *Journal of Hospitality, Leisure, Sport & Tourism Education*, vol. 28, p. 100312, 2021. https://doi.org/10.1016/j.jhlste.2021.100312
[17] M. R. Kartamiharja, W. Sopandi, and D. Anggraeni, "Implementation of problem-based learning (PBL) approach in chemistry instructional with context of tofu liquid waste treatment," *International Journal of Learning, Teaching and Educational Research*, vol. 19, no. 5, pp. 47-77, 2020. https://doi.org/10.26803/ijlter.19.5.4
[18] Z. Ismuwardani, A. Nuryatin, and M. Doyin, "Implementation of project based learning model to increased creativity and self-reliance of students on poetry writing skills," *Journal of Primary Education*, vol. 8, no. 1, pp. 51-58, 2019.
[19] D. Sumardi, I. L. Mursyidah, E. S. W. Jannah, and Z. R. Ridlo, "The implementation of RBL-STEAM with pictorial riddle and augmented reality in improving students' historical thinking skill," *Edelweiss Applied Science and Technology*, vol. 8, no. 4, pp. 213-237, 2024. https://doi.org/10.55214/25768484.v8i4.1163
[20] Z. R. Ridlo and J. Waluyo, "Exploring the impact of makerspace-based learning materials on students' computational thinking skills: Using machine learning to address challenges in smart coffee agroforestry," *Edelweiss Applied Science and Technology*, vol. 8, no. 5, pp. 52-73, 2024. https://doi.org/10.55214/25768484.v8i5.1630
[21] B. Wahono, P.-L. Lin, and C.-Y. Chang, "Evidence of STEM enactment effectiveness in Asian student learning outcomes," *International Journal of STEM Education*, vol. 7, no. 1, pp. 1-18, 2020.
[22] D. Jumiarni, S. Irawati, E. W. Winarni, and F. A. Yanti, "Development of biotechnology module based on integrated project based learning," in *Proceedings of the Online Conference of Education Research International (OCERI 2023) Atlantis Press SARL*, 2024, doi: https://doi.org/10.2991/978-2-38476-108-1.
[23] A. P. Utomo, L. Hasanah, S. Hariyadi, E. Narulita, and N. Umamah, "The effectiveness of STEAM-based biotechnology module equipped with flash animation for biology learning in high school," *International Journal of Instruction*, vol. 13, no. 2, pp. 463-476, 2020. https://doi.org/10.29333/iji.2020.13232a
[24] U. Sarı, E. Duygu, Ö. F. Şen, and T. Kırındı, "The effects of STEM education on scientific process skills and STEM awareness in simulation based ınquiry learning environment," *Journal of Turkish Science Education*, vol. 17, no. 3, pp. 387-405, 2020. https://doi.org/10.36681/tused.2020.34
[25] C.-C. Hu, H.-C. Yeh, and N.-S. Chen, "Enhancing STEM competence by making electronic musical pencil for non-engineering students," *Computers & Education*, vol. 150, p. 103840, 2020. https://doi.org/10.1016/j.compedu.2020.103840
[26] L. D. English, "STEM education K-12: Perspectives on integration," *International Journal of STEM Education*, vol. 3, no. 1, p. 3, 2016. https://doi.org/10.1186/s40594-016-0036-1
[27] R. Dafik, Z. R., R. Huzaimah, I. M. Tirta, and R. Nisviasari, "The analysis of the implementation of research-based learning with STEM approach to improving the students' metaliteracy in solving the resolving strong dominating set problem on traffic CCTV placement," *International Journal of Current Science Research and Review*, vol. 5, no. 8, 2022. https://doi.org/10.47191/ijcsrr/v5-i8-37
[28] R. Adawiyah *et al.*, "Enhancing students' combinatorial thinking for graceful coloring problem: A STEM-based, research-informed approach in ATM placement," *Tadris: Jurnal Keguruan dan Ilmu Tarbiyah*, vol. 8, no. 1, pp. 77-93, 2023.
[29] A. M. H. Ahkam, Dafik, and A. I. Kristiana, "The development of RBL-STEM learning materials to improve students' combinatorial thinking skill in solving local (a,d)-edge antimagic coloring problems for line motif batik design," *World Journal of Advanced Research and Review*, vol. 21, no. 2, pp. 276–285, 2024. https://doi.org/10.30574/wjarr.2024.21.2.0212
[30] Y. N. N. Aziz, Dafik, and I. M. irta, "The development of RBL-STEM learning materials to improve students' combinatorial thinking skills in solving b-coloring problems for companion farming," *World Journal of Advanced Research and Review*, vol. 21, no. 1, pp. 2225–2232, 2024. https://doi.org/10.30574/wjarr.2024.21.1.0100
[31] D. Irawan, D. Dafik, and I. M. Tirta, "The development of RBL-STEM learning materials to improve students' computational thinking skills in solving rainbow vertex antimagic coloring problems and its application for batik motif design," *International Journal of Current Science Research and Review*, vol. 6, no. 7, pp. 5294–5304, 2023. https://doi.org/10.47191/ijcsrr/v6-i7-144
[32] S. T. Thiagarajan, A. Dorothy, and M. I. Somme!, "Instructional development for training teachers of exceptional children: A sourcebook," *Journal of School Psychology*, vol. 14, no. 1, p. 75, 1974.
[33] D. Marsidi, Susanto, and A. I. Kristiana, "The development of RBL-STEM learning materials to improve students' computational thinking skills in solving convolutional neural network problems," *World Journal of Advanced Research and Review*, vol. 21, no. 1, pp. 2373–2381, 2024. https://doi.org/10.30574/wjarr.2024.21.1.0219
[34] Z. R. Ridlo, Dafik, and C. I. W. Nugroho, "Report and recommendation of implementation research-based learning in improving combinatorial thinking skills embedded in STEM parachute design activities assisted by CCR (cloud classroom)," *Universal Journal of Educational Research*, vol. 8, no. 4, pp. 1413–1429, 2020. https://doi.org/10.13189/ujer.2020.080434
[35] R. S. D. Gita, J. Waluyo, and I. Dafik, "On the shrimp skin chitosan STEM education research-based learning activities: Obtaining an alternative natural preservative for processed meat," presented at the IOP Conference Series: Earth and Environmental Science, 747(1), 012123. https://doi.org/10.1088/1755-1315/747/1/012123, 2021.

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No: 2575/UN25.5.1/LL/2023. 05 April 2023

Ahdatu Uli Khikamil Maulidiya
Department of Science Education, Faculty of Education, University of Jember

The Social Research Ethics Commission, Faculty of Education, University of Jember has recently reviewed your research proposal outlined below. After careful assessment, we are pleased to grant full ethical approval of the research.

Project title	:	The Development of RBL-STEM Learning Materials to Improve the Students' Combinatorial Thinking Skills in Solving Transgenic Sugarcane Confirmation Problem Using PCR Technique
Approval date	:	06 April 2023
Expiry date	:	06 April 2024

The above project must be conducted strictly in accordance with the proposed project that was submitted and granted ethic approval. The researchers are advised to immediately make a submission for approval of amendments of the approved project. It is important to note that your project must comply the following regulation:

1. Ministry of Research and Technology, decree No. 25/M/Kp/III/2013 on Research ethics
2. Indonesian Institute of Science, decree No. 06/E/2013 on Research ethics
3. University of Jember, Research ethics and publication

You can now commence your research project and we wish you all the best for the conduct of the research project.

Social Research Ethics Commission
Faculty of Education, University of Jember
Vice Dean Academic Affairs

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Nurman, Ph.D